A STUDY OF CHICKPEA (*CICER ARIETINUM* L.) SEED STARCH CONCENTRATION, COMPOSITION AND ENZYMATIC HYDROLYSIS PROPERTIES

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfilment of the Requirements

For the Degree of Doctor of Philosophy

In the Department of Plant Sciences

University of Saskatchewan

Saskatoon, Saskatchewan

By

Adams Frimpong

© Copyright Adams Frimpong, September 2010. All rights reserved

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in thesis in whole or part should be addressed to:

Head of the Department of Plant Sciences
51 Campus Drive
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5A8

ABSTRACT

Grain quality in chickpea (*Cicer arietinum* L.) is a major factor affecting its consumption for human nutrition and health benefits. Some of the major factors affecting chickpea grain quality are: seed weight, size, colour, protein, starch and amylose concentration, and amylopectin structure. The objectives of this study were to: 1) determine variation, repeatability and genotype by environment interaction on thousand seed weight, starch, amylose and protein concentration of chickpea cultivars adapted to western Canada; 2) assess variations in global chickpea germplasm for thousand seed weight, seed size, protein, starch and amylose concentrations; and 3) characterize the desi and kabuli type chickpea for starch concentration, composition, and amylopectin structure to study their effect on starch enzymatic hydrolysis. Limited variation was observed in seed composition of chickpea cultivars adapted to the western Canadian prairies. Significant genotype by environment interaction occurred for starch, amylose, and protein (except for kabuli) concentrations, seed yield and thousand seed weight indicating that testing over a wide range of environments is needed to identify genotypes for grain quality improvement. Repeatability of starch, amylose, and protein concentrations was low and inconsistent across chickpea market classes. Broad sense heritability was higher than repeatability across all traits for all market classes implying that repeatability estimates do not set upper limits to heritability if significant genotype by environment interaction is present. The negative relationship between seed constituents and yield indicates that selection for chickpea cultivars with desired seed composition may require compromise with yield and indirect selection. All the mini core accessions that had above average seed diameter score in both desi and kabuli also had above average score for thousand seed weight. Selecting mini core with promising intrinsic and extrinsic quality characteristics may reduce yield. Slowly digestible starch was negatively correlated with hydrolysis index in both pure starch and meal starch of desi and kabuli. Amylose had a strong relationship with resistant starch but not with rate of starch hydrolysis. Genotypes with a significantly higher rate of starch hydrolysis had significantly lower 60-80 μm starch granule size volume. Amylopectin B2 chains were related to slowly digestible starch of meal (except kabuli) and extracted starch. Resistant starch positively correlated with B1 fraction of amylopectin chain length in both desi and kabuli meal starch. Our results suggest that there is no major difference between starch composition in the two chickpea

market classes, although only three genotypes of each class were tested. The meal components affect the starch hydrolytic properties and the effect is genotype specific. The results also show that amylopectin structure influences starch hydrolytic properties. These observations emphasize that complete characterization of seed components is needed to obtain meaningful results regarding the desired nutritional and health benefits attributed to any grain.

ACKNOWLEDGEMENTS

I am grateful to my supervisor and mentor Dr. Ravindra N. Chibbar for his guidance, encouragement and trust through out this research project. My gratitude is extended to my advisory committee members: Drs. Bunyamin Tar'an, Bruce Coulman, Rosalind Bueckert and Nicholas Low for their advice and constructive suggestions.

Special thanks go to Dr. Thomas Warkentin for providing chickpea materials for a part of this study. Drs. P. M. Gaur and H.D. Upadhyaya [International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, Andhra Pradesh, India] are gratefully acknowledged for providing the chickpea minicore collection used in this study. I appreciate professional advice from Drs. Monica Baga and Pooba Ganeshan as well as the technical support from the Grain Molecular Quality Laboratory.

I acknowledge financial support from Canadian Commonwealth Scholarship, Canada Research Chairs program, Canada Foundation for Innovation and Department of Plant Sciences (Bruce Coulman) for this study.

The Council for Scientific and Industrial Research and the Government of Ghana are gratefully acknowledged for granting study leave for my PhD studies.

I am humbled by the unflinching support and encouragement from my parents, siblings and the entire family. I will like to express my deep and sincere appreciation and thanks to my wife Rehaina, and the children Ankoana, Afriyie and Naana for their sacrifice and support during my studies. To God be the Glory, for He is the source of all wisdom.

TABLE OF CONTENTS

PERMISSION TO USE i
ABSTRACT ii
ACKNOWLEDGEMENT iv
TABLE OF CONTENTS v
LIST OF TABLES ix
LIST OF FIGURES xii
LIST OF ABBREVIATIONS xvi
Chapter 1: Introduction 1
Chapter 2: Literature review 4
2.1 Chickpea 4
2.1.1 Taxonomy 4
2.1.2 Classification 4
2.1.3 Origin and domestication 5
2.1.4 Distribution 7
2.2 Utilization of chickpea 8
2.2.1 Human food 8
2.2.2 Human health benefits 8
2.2.3 Animal feed 9
2.3 Chickpea germplasm 10
2.3.1 Germplasm collection 10
2.3.2 Mini core 10
2.4 Chickpea seed composition 13
2.5 Carbohydrates 13
2.5.1 Monosaccharides 13
2.5.2 Disaccharides 13
2.5.3 Oligosaccharides 14
2.5.4 Polysaccharides 14
2.6 Starch 14
2.6.1 Starch granule structure and organization 19

2.6.2 Starch Biosynthesis 22
2.7.1 Seed starch isolation methods 23
2.7.2 Total starch determination methods 24
2.7.3 Amylose determination methods 25
2.7.4 Amylopectin chain length determination methods 26
2.8.1 Resistant starch 27
2.8.2 Resistant starch measurement 29
2.8.3 Factors affecting amount of resistant starch and rate of hydrolysis 30
2.8.4 Relationship between starch composition and structure with RS 30
2.9 Genotype by environment interaction and chickpea chemical Composition 31
2.10 Repeatability and heritability 34
2.11 Hypothesis for thesis 38
Chapter 3: Genotype and growing environment influence chickpea seed Composition 40
3.1 Abstract 40
3.2 Introduction 40
3.3 Materials and methods 42
3.3.1 Varieties and environments 42
3.3.2 Seed weight 43
3.3.3 Dehulling of desi and grinding of chickpeas 43
3.3.4 Total starch 43
3.3.5 Isolation of starch granules 46
3.3.6 Amylose determination 46
3.3.7 Protein concentration 47
3.3.8 Statistical analyses 47
3.3.9 Repeatability estimates 48
3.3.10 Heritability estimates 48
3.4 Results and discussions 48
3.4.1 Desi chickpea 49
3.4.2 Kabuli chickpea 52
3.4.3 Trait correlations 54

3.4.4 Repeatability and heritability 64
3.5 Conclusions 77
Chapter 4: Variation in chickpea mini core collection for seed quality Traits 78
4.1 Abstract 78
4.2 Introduction 78
4.3 Materials and methods 80
4.3.1 Plant materials 80
4.3.2 Growing sites and conditions 81
4.3.3 Extrinsic characters 81
4.3.3.1 Thousand seed weight 81
4.3.3.2 Seed diameter 81
4.3.3.3 Seed coat colour 82
4.3.4 Intrinsic quality determinations 82
4.3.4.1 Starch concentrations 82
4.3.4.2 Amylose concentrations 82
4.3.4.3 Protein concentrations 82
4.3.5 Data analysis 82
4.4. Results and discussions 83
4.4.1 Mini core collection 83
4.4.2 Extrinsic characters –seed colour, shape and size 83
4.3.3 Intrinsic seed characteristics – starch, amylose and protein Concentration 89
4.4.4 Trait correlations 91
4.4.5 Shannon-Weaver Diversity index 93
4.4.6 Comparison of the diversity in primary and secondary centres of Diversity 96
4.5 Conclusions 96
Chapter 5: Chickpea starch structure and its relationship to starch hydrolytic activities in extracted starch and seed meal 98
5.1 Abstract 98
5.2 Introduction 98
5.3 Materials Methods 100

5.3.1 Genotypes and growing conditions 100
5.3.2 Dehulling and grinding of chickpeas 100
5.3.3 Isolation of starch granules 100
5.3.4 Amylose determination 102
5.3.5 Starch granule size analysis 102
5.3.6 Determination of the α- 1,4-glucan chain-length distribution by capillary electrophoresis 103
5.3.7 Resistant starch determination 103
5.3.7.1 Starch hydrolysis 103
5.3.7.2 Measurement of non-hydrolyzed starch 104
5.3.7.3 Measurement of hydrolyzed starch 104
5.3.7.4 Calculations 105
5.3.7.5 Determination of starch hydrolysis index 105
5.3.8 Statistical analysis 106
5.4 Results and discussions 106
5.4.1 Screening chickpea genotypes for starch hydrolytic activity 106
5.4.2 Total starch and amylose concentration 111
5.4.3 Starch granule size 111
5.4.4 Amylopectin chain length 114
5.4.5 Rate of starch hydrolysis 114
5.4.6 Starch digestibility studies and hydrolysis index (*HI*) 117
5.4.7 Desi and kabuli starch characteristics 120
5.4.8 Relationship between starch hydrolytic activities and starch characteristics in chickpea meal and extracted starch 121
5.5. Conclusions 128
Chapter 6: General discussion and conclusions 129
6.1 Future research directions 133
6.2 Conclusions 134
LITERATURE CITED 135

LIST OF TABLES

Table 2.1	Chickpea accessions collected and housed at ICRISAT	12
Table 2.2	Carbohydrate concentration (%) of some pulse seeds	15
Table 2.3	Comparison of carbohydrate composition of desi and kabuli chickpea whole seeds	16
Table 2.4	Comparison of protein and amino acid composition of desi and kabuli chickpea whole seeds	17
Table 2.5	Comparison of mineral composition and concentration (mg $100g^{-1}$)	18
Table 2.6	Classification of starch, its structure and physiological properties	28
Table 2.7	Chickpea g x e studies on agronomic traits published since 1971 and organized by year of publication and methodology employed for analysis of the G x E interaction	36
Table 3.1	Soil zone, monthly precipitation (P, mm) and mean temperature (T,$^{\circ}$C) during growing period at trial locations	44
Table 3.2	Description of chickpea varieties used in the study	45
Table 3.3	Mean squares of combined ANOVA and coefficient of variation (CV) for seed yield, seed weight and protein, starch and amylose concentrations of desi and kabuli chickpea varieties grown in different environments in western Canada.	50
Table 3.4	Seed yield, seed weight and protein, starch and amylose concentrations of desi chickpea varieties evaluated across different environments in western Canada	51
Table 3.5	Seed yield, seed weight and protein, starch and amylose concentrations of kabuli chickpea varieties evaluated across environments in western Canada.	56
Table 3.6	Correlation coefficients among chickpea seed yield, seed weight and protein, starch and amylose concentrations evaluated across environments in western Canada	57
Table 3.7	Repeatability (r_{approx}) estimates for seed yield, seed weight and protein, starch and amylose concentrations of desi and	76

	kabuli chickpea varieties evaluated across environments in western Canada	
Table 3.8	Heritability ($H_{broad\ sense}$) estimates for seed yield, seed weight, protein, starch, and amylose concentrations of desi and kabuli chickpea varieties evaluated across environments in western Canada.	76
Table 4.1	Representation of chickpea mini core (number of accessions) by region and type as shown in Figure 1	85
Table 4.2	Mean/range and standard deviation for seed diameter, seed weight and colour measurement of the chickpea min core collection in 2006 and 2007 evaluated at Kyle, SK, Canada	88
Table 4.3	Mean/range and standard deviation for grain starch, amylose and protein concentration of chickpea min core collection in 2006 and 2007 evaluated at Kyle, SK, Canada	90
Table 4.4	Correlation coefficients among six quality traits in desi mini core of 2006 and 2007 growing season in Kyle, Canada	92
Table 4.5	Correlation coefficients among six quality traits in kabuli mini core of 2006 and 2007 growing season in Kyle, Canada	92
Table 4.6	Shannon-Weaver diversity index for chickpea mini core (desi genotypes)	94
Table 4.7	Shannon-Weaver diversity index for chickpea mini core (kabuli genotypes)	94
Table 4.8	Comparison of Shannon-Weaver diversity index at the primary (region 2) and secondary (region 3) centres of chickpea diversity (desi genotypes)	95
Table 4.9	Comparison of Shannon-Weaver diversity index at the primary (region 2) and secondary (region 3) centres of chickpea diversity (kabuli genotypes)	95
Table 5.1	Description and origin of selected chickpea genotypes used to analyze seed meal hydrolytic activity.	101
Table 5.2	Mean ±sd and range of starch and amylose concentration in selected chickpea mini core genotypes	109
Table 5.3	Amylopectin chain length distribution in amylopectin fraction of selected chickpea genotypes.	110

Table 5.4	Rate of hydrolysis (mg glucose solubilized per hour) of desi chickpea meal at 20, 80, 120, and 240 minutes of incubation with pancreatic α-amylase.	115
Table 5.5	Rate of hydrolysis (mg glucose solubilized per hour) of desi chickpea extracted starch at 20, 80, 120, and 240 minutes of incubation with pancreatic α-amylase.	115
Tab*l*e 5.6	Rate of hydrolysis (mg glucose solubilized per hour) of kabuli chickpea meal at 20, 80, 120, and 240 minutes of incubation with pancreatic α-amylase	116
Table 5.7	Rate of hydrolysis (mg glucose solubilized per hour) of kabuli chickpea extracted starch at 20, 80, 120, and 240 minutes of incubation with pancreatic α-amylase	116
Table 5.8	Digestibility of starch and starch fractions from meal of desi chickpea genotypes	118
Table 5.9	Digestibility of starch and starch fractions from extracted starch of different desi chickpea genotypes	118
Table 5.10	Digestibility of starch and starch fractions from meal of kabuli chickpea genotypes	119
Table 5.11	Digestibility of starch and starch fractions from extracted starch of different kabuli chickpea genotypes	119
Table 5.12	Summary of starch hydrolytic activity in meal/extracted starch and amylopectin structure in selected chickpea genotypes used for analyses presented in Figures 5.5 to 5.11.	122

LIST OF FIGURES

Figure 2.1	Picture showing: (a) desi flowered plant and dried seeds (b) kabuli flowered plant and dried seeds.	6
Figures 2.2	Amylose (top) and amylopectin (bottom) linear structures	20
Figure 2.3	Branching pattern of amylopectin schematically representing α 1-4 chains (A, B1-B3) joined by 1-6 α-linkage branch points	20
Figure 2.4	Overview of the organization of starch granule structure. From lowest level (top) of hard crystalline and soft amorphous/semicrystalline shells of the granule to the highest level (bottom) of crystal structure types A and B.	21
Figure 3.1	Plots of first two major principal components for genotypic effect on major seed components in desi chickpeas, representing genotype-focused, singular value partitioning for amylose: 1, ICC-12512-1; 2, CDC Cabri; 3, Myles; 4, CDC Anna; 5, Vanguard; 6, ICC-12512-9; 7, 316B-42.	58
Figure 3.2	Plots of first two major principal components for genotypic effect on major seed components in desi chickpeas, representing genotype-focused, singular value partitioning for starch: 1, ICC-12512-1; 2, CDC Cabri; 3, Myles; 4, CDC Anna; 5, Vanguard; 6, ICC-12512-9; 7, 316B-42.	59
Figure 3.3	Plots of first two major principal components for genotypic effect on major seed components in desi chickpeas, representing genotype-focused, singular value partitioning for protein: 1, ICC-12512-1; 2, CDC Cabri; 3, Myles; 4, CDC Anna; 5, Vanguard; 6, ICC-12512-9; 7, 316B-42.	60
Figure 3.4	Plots of first two major principal components for environmental effect on major seed components in desi chickpeas, representing environment focused, singular value partitioning for amylose: 1, Bow Island; 2, Brooks; 3, Davidson; 4, Elrose; 5, Goodale; 6, Kyle 2004; 7, Kyle 2005; 8, Scott 2004; 9, Scott 2005; 10, Swift Current.	61
Figure 3.5	Plots of first two major principal components for environmental effect on major seed components in desi chickpeas, representing environment focused, singular value partitioning for starch: 1, Bow Island; 2, Brooks; 3, Davidson; 4, Elrose; 5, Goodale; 6, Kyle 2004; 7, Kyle 2005; 8, Scott 2004; 9, Scott 2005; 10, Swift Current.	62

Figure 3.6 Plots of first two major principal components for environmental effect on major seed components in desi chickpeas, representing environment focused, singular value partitioning for protein: 1, Bow Island; 2, Brooks; 3, Davidson; 4, Elrose; 5, Goodale; 6, Kyle 2004; 7, Kyle 2005; 8, Scott 2004; 9, Scott 2005; 10, Swift Current. 63

Figure 3.7 Plots of first two major principal components for genotypic effect on major seed components in kabuli chickpeas, representing genotype focused, singular value partitioning for amylose: 1, Amit; 2, CDC Frontier; 3, CDC Xena; 4, FLIP97-45C; 5, FLIP98-134C; 6, FLIP97-133C; 7, FLIP98-135C; 8, 97-Indian2-1; 9, Sanford. 65

Figure 3.8 Plots of first two major principal components for genotypic effect on major seed components in kabuli chickpeas, representing genotype focused, singular value partitioning for starch: 1, Amit; 2, CDC Frontier; 3, CDC Xena; 4, FLIP97-45C; 5, FLIP98-134C; 6, FLIP97-133C; 7, FLIP98-135C; 8, 97-Indian2-1; 9, Sanford. 66

Figure 3.9 Plots of first two major principal components for genotypic effect on major seed components in kabuli chickpeas, representing genotype focused, singular value partitioning for protein: 1, Amit; 2, CDC Frontier; 3, CDC Xena; 4, FLIP97-45C; 5, FLIP98-134C; 6, FLIP97-133C; 7, FLIP98-135C; 8, 97-Indian2-1; 9, Sanford. 67

Figure 3.10 Plots of first two major principal components for environmental effect on major seed components in kabuli chickpeas, representing environment-focused, singular value partitioning for amylose: 1, Bow Island; 2, Davidson; 3, Elrose; 4, Goodale; 5, Hodgeville; 6, Kyle; 7, Swift Current. 68

Figure 3.11 Plots of first two major principal components for environmental effect on major seed components in kabuli chickpeas, representing environment-focused, singular value partitioning for starch: 1, Bow Island; 2, Davidson; 3, Elrose; 4, Goodale; 5, Hodgeville; 6, Kyle; 7, Swift Current. 69

Figure 3.12 Plots of first two major principal components for environmental effect on major seed components in kabuli chickpeas, representing environment-focused, singular value partitioning for protein: 1, Bow Island; 2, Davidson; 3, Elrose; 4, Goodale; 5, Hodgeville; 6, Kyle; 7, Swift Current. 70

Figure 3.13 Trait-focused, singular value partitioning PCA plots indicating genetic correlations among five traits (S, starch; P, protein; Am, amylose; K, seed weight; Y, seed yield) in kabuli chickpea: genotype-focused, singular value partitioning for 71

kabuli class

Figure 3.14	Trait-focused, singular value partitioning PCA plots indicating genetic correlations among five traits (S, starch; P, protein; Am, amylose; K, seed weight; Y, seed yield) in kabuli chickpea: environment-focused, singular value partitioning for kabuli class	72
Figure 3.15	Trait-focused, singular value partitioning PCA plots indicating genetic correlations among five traits (S, starch; P, protein; Am, amylose; K, seed weight; Y, seed yield) in desi chickpea: genotype-focused, singular value partitioning for desi class	73
Figure 3.16	Trait-focused, singular value partitioning PCA plots indicating genetic correlations among five traits (S, starch; P, protein; Am, amylose; K, seed weight; Y, seed yield) in desi chickpea: environment-focused, singular value partitioning for desi class	74
Figure 4.1	Centers of origin and diversity for chick (*Cicer arietinum* L.)	84
Figure 4.2	Variations of Colour in chickpea mini core	86
Figure 4.3	Variations of seed shape in chickpea mini core	86
Figure 5.1	Rate of meal starch hydrolysis of selected desi chickpea grown across three environments in Western Canada	107
Figure 5.2	Rate of meal starch hydrolysis of selected kabuli chickpeas grown across three environments in Western Canada.	108
Figure 5.3	Starch granule size distribution in selected desi chickpea genotypes.	112
Figure 5.4	Starch granule size distribution in selected kabuli chickpea genotypes.	113
Figure 5.5	Average linkage and correlation coefficient distance in characteristics of starch extracted from selected chickpea genotypes	123
Figure 5.6	Average linkage and correlation coefficient distance among chickpea genotypes based on extracted starch characteristics	123
Figure 5.7	Average linkage and correlation coefficient distance in amylopectin chains based on extracted starch from selected chickpea genotypes	124
Figure 5.8	Average linkage and correlation coefficient distance among chickpea genotypes based on amylopectin structure of	124

extracted starch

Figure 5.9 Average linkage and correlation coefficient distance in starch hydrolytic activities and starch characteristics based on extracted starch from selected chickpea genotypes 125

Figure 5.10 Average linkage and correlation coefficient distance in starch hydrolytic activities and starch characteristics based on meal prepared from selected chickpea genotypes 125

Figure 5.11 Average linkage and correlation coefficient distance among chickpea genotypes based on meal starch hydrolytic activities and starch characteristics 126

LIST OF ABBREVIATIONS

AMG	Amyloglucosidase
AM	Amylose
AUC	Area under the curve
A	A-chains (DP6-12) of amylopectin chains
B1	B1-chains (DP13-25) of amylopectin chains
B2	B2-chains (DP26-35) of amylopectin chains
C	C –chains (DP36-55) of amylopectin chains
DMSO	Dimethyl sulfoxide
DMRT	Duncan multiple range test
DP	Degree of polymerization
GOPOD	Glucose oxidase per oxidase
HPLC	High performance liquid chromatography
HP-SEC	High performance size exclusion chromatography
HI	Hydrolysis index
IMS	Industrial methylated spirit
MOPS	3-(N-morpholino) propanesulfonic acid
PCA	Principal component analysis
P	Protein
SDS	Slowly digestible starch
SGS5	Starch granule size ranging from 5-20µm
SGS20	Starch granule size ranging from 20-40µm
SGS40	Starch granule size ranging from 40-60µm

SGS60	Starch granule size ranging from 60-80µm
RDS	Rapidly digestible starch
RS	Resistant starch
ST	Starch (total)
TSW	Thousand seed weight

CHAPTER 1

INTRODUCTION

Pulse, coined from the latin word *puls* meaning thick soup, is the dried edible seed of fabaceae (legume) family members such as chickpea, pea, lentil, and dry beans. In 2007 global production and area seeded to pulse was estimated to be 60.9 million tonnes and 74.13 million ha, respectively (FAO, 2009). Pulse production in the Canadian prairie dates back to 1883, however, pulse crops began playing significant economic role in western Canada in the 1970s when the Canadian wheat oversupply urged farmers to diversify into crops like pea, lentil and rape seed (Saskatchewan Pulse Growers, 2009; Carlyle, 2004). Global chickpea production and area under cultivation has been estimated to be 8.8 million tonnes and 11.6 million ha, respectively (Food and Agriculture Organization, 2009), accounting for 12% of global pulse production and is third after beans and pea. Chickpea (*Cicer arietinum* L.) was commercially produced in Western Canada during the 1993/1994 production season, to serve as an inexpensive protein source and health food, with two market classes: kabuli and desi adapted to brown, and brown and dark brown soil zones, respectively (Pulse Canada, 2009). Presently, Canada accounts for 2.3%, 8.6% and 9.2% of global chickpea production, trade volume and export value, respectively (FAO, 2009). Saskatchewan accounts for >80% of the Canadian chickpea production (Pulse Canada, 2009).

The nutritive value of pulses as protein and carbohydrate source was recognized almost 10,000 years ago. Pulse crops including chickpea are a staple food globally playing critical dietary roles among vegetarians (Wood and Grusak, 2007). Pulses are a good source of starch, protein (2-3 times higher than cereals), fibre, minerals, vitamins and phytochemicals. Pulse proteins are rich in the essential amino acid lysine, which is deficient in cereal grains, thus making pulses a good nutritional compliment to cereals. The principal nutritional component in chickpea is carbohydrate constituting 51-65% in desi types and 54-71% in kabuli types (Wood and Grusak, 2007). All the main carbohydrates, such as monosaccharides, disaccharides, oligosaccharides and polysaccharides are present in chickpea seeds. The major storage carbohydrate in chickpea seed is starch and it accounts for 30-57% of seed dry weight. Amylose makes up 20-42% and 21-46.5% of desi and kabuli starch, respectively (Wood and Grusak, 2007).

Starch is also classified based on digestibility as rapidly digestible starch (RDS), slowly digestible starch (SDS) and indigestible or resistant starch (RS). Unlike RDS and SDS, resistant starch is not digested in the small intestine but fermented in the large intestine by bifidobacteria into short chain fatty acids (Englyst et al., 1992a; Topping and Clifton, 2001). Short chain fatty acids are simple carboxylic acids (eg butyrate: CH_3-CH_2-CH_2-COOH) which lower colonic pH thereby facilitating mineral absorption and promote natural death rate of cancer cells, protecting colon lining from cancer polyps (Topping and Clifton, 2001). Lower colonic pH also stimulates excretion of protein metabolites, which are potent carcinogens (Binghams, 1990). Phillips et al. (1995) reported in clinical studies that consumption of 39g resistant starch per day over three weeks reduced fecal pH from 6.9 to 6.3 and increased the levels of acetate (by 38% mmol/day) and butyrate (by 100 mmol/day).

Legume starch (including chickpea) is less digestible than cereal starch probably due to high amylose, starch granule structure differences and cell wall components blocking enzyme access to granules (Carre et al., 1998). Roasted chickpeas and green bananas have been reported as the richest sources of resistant starch (Muir and O'Dea, 1992). In high amylose maize (Hi-Maize), a strong relationship between increasing amylose concentration and amount of resistant starch has been reported (Brown, 1996). Consumer acceptance and consumption of Hi-Maize bread in Australia has increased by 1.4% (Brown, 1996). It is possible that high amylose chickpea bread (chapatti) will also increase chickpea consumption. Despite its economic and nutritional roles, chickpea research and improvement in Canada has in the past focused on enhancing yield (Vandenberg et al., 2004; Slinkard et al., 2000; Anbessa et al., 2006) and improving agronomic practices (Gan et al., 2007).

In the Canadian prairies, chickpea production is sole cropping, rotational with wheat and mainly rain fed with some supplemental irrigation during periods of drought. Chickpea is a dry land crop, because it has a deep root system that allows for extraction of soil moisture from lower soil profile (Sekhon and Singh, 2007). Chickpea yield is increased with early season single irrigation of 25mm while later irrigation decreased yield and extended maturity period thus risking frost damage in Saskatchewan (AAFC, 2009). Chickpea seed quality improvement is challenging as seed composition is affected by environment, agronomic practices and genetic factors (Wood and Grusak, 2007). Chickpea seed quality can be extrinsic or intrinsic. The

extrinsic component reflects the appearance of seed, while intrinsic component is governed by the seed composition.

As a first step towards chickpea seed composition improvement in western Canada, it is important to characterize the extrinsic characteristics and selected seed constituents in currently grown chickpea varieties. To develop breeding strategies for seed quality improvement it is imperative to study the genotype x environment interactions and repeatability of selected seed quality traits. There are limited published reports about chickpea seed composition, but no systematic study to characterize genetic variation that exists in chickpea gene pool. Although chickpea derived food products have been shown to have low glycemic indices and increased amounts of resistant starch, there are no systematic studies to associate these characteristics with chickpea seed composition. The main objective of this research is to characterize chickpea seed composition and understand the influence of genotype and environment on deposition of seed starch and its composition. Selected global chickpea germplasm will be analyzed for genetic variation in seed protein and starch composition. Attempts will aso be made to study the difference in desi and kabuli chickpea cultivars seed starch composition, structure and associate these properties with digestibility using an in vitro enzymatic assay. Successful completion of this project will result in a better understanding of chickpea seed composition and strategy (ies) for chickpea seed quality improvement. The specific objectives of this study were to:

1. Study variations, repeatability and genotype by environment interaction on thousand seed weight, starch, amylose and protein concentrations of chickpea adapted to Saskatchewan’s environment

2. Assess variations in global chickpea minicore for thousand seed weight, protein, starch and amylose concentrations

3. Characterize selected chickpea genotypes for starch composition and structure and study their effect on starch enzymatic hydrolysis.

CHAPTER 2

LITERATURE REVIEW

2.1. Chickpea

2.1.1. Taxonomy

Chickpea is a self pollinating, diploid (2n=2x=14, 16, 24, 32, 33) pulse crop with genome size 1C=740 Mbp (van der Maesen et al., 2007). Cultivated chickpea belongs to the genus Cicer, tribe *Cicereae* Alef., family *Leguminosae* and sub-family *Papilionoideae* (van der Maesen et al., 2007). The genus *Cicer* comprises of 44 species divided into two subgenera *Pseudononis* Popov and *Viciastrum* Popov; four sections – Monocier Popov, *Chamaecicer* Popov, *Polycicer* Popov, and *Acanthocicer* Popov; and 14 series (Popov 1929; van der Maesen, 1972). The section *Monocicer* is made up of annual species and is subdivided into three series; Arietina (imparipinnate leaves with none to small arista or bract), cirrhifera (leaves ending in tendrils with short arista) and macro-arista (leaves imparipinnate, long arista). Only one of the 44 known species of the genus *Cicer, C. arietinum* L. is cultivated in 49 countries worldwide with Asia and Africa accounting for 96% of the global production (van der Maesen et al., 2007). *C. echinospermum* and *C. reticulatum* form the primary gene pool with the cultivated *C. arietinum L.* However, *C. bijugum, C. judaicum,* and *C. pinnatifidum* form the secondary gene pool while other *Cicer* species form the tertiary gene pool.

2.1.2 Classification

There are two types of chickpea: kabuli and desi types (van der Maesen, 1972; Figure 1). Morphologically, desi and kabuli types are distinct with intermediate forms rarely appearing (Iruela et al., 2002). Kabuli, the 'macrosperma' types have relatively lager pods, seeds, leaves, smoother and rounder thin seed coat, pale white to cream-coloured seeds and taller in stature (van der Maesen, 1972) compared to desi. Desi types have small, dark seeds and a rough coat with a pronounced angularity and strongly ridged surface (van der Maesen, 1972). Kabuli types are less wrinkled. Seed coat of desi types is considerably thicker than that of kabuli types but in both types there is good adherence of seed coat to the cotyledons (Knights, 1980). Desi and kabuli are broadly representative of single gene pool within chickpea hence easily crossable. However, they have evolved in separate regions under different environmental conditions and

subjected to different selection pressures. Kabuli requires more rainfall than desi. Nutritional differences between desi and kabuli seeds might be attributable to seed type and/or the combined effects of natural and artificial selection pressure (Saini and Knights, 1984).

2.1.3 Origin and domestication

Chickpea is an ancient low-input pulse crop of modern times. It has been cultivated for over 9,500 years in the Fertile Crescent from Turkey to Iran, since the beginning of agriculture (Yadav et al., 2007), along with other domesticates to meet man's basic needs: carbohydrates, protein, vegetable, animal fibre, animal transport and traction (Redden and Berger, 2007),

As part of the evolution of agriculture over 10,000 years ago when annuals were first planted along the shores of the expanded lakes and ponds in the Levant (Bar-Yosef, 1998), chickpea was domesticated (Abbo et al., 2003). Chickpea originated in the southern Caucasus and northern Persia (van der Maesen 1987). Linguistic evidence suggests that Kabuli type entered India through Kabul, capital of Afghanistan some two centuries ago and got the name "kabuli chana" in Hindi (van der Maesen, 1972). However, Ladizinsky, (1975) reported the center of origin to be southeastern Turkey.

Serret et al., (1997) analyzed 30 accessions of chickpeas from 11 different countries using restriction fragment length polymorphism (RFLP) and concluded that there are three centers of diversity for chickpea: Pakistan-Afghanistan, Iraq-Turkey and Lebanon, while India previously considered as a secondary center of diversity for chickpea, showed lower diversity than the above regions. Numerous plant species were domesticated and harnessed in the Near East about 11,000 years ago (Kareem et al., 2007). Meanwhile Zohary (1989) in his analysis showed that pulses including chickpea were domesticated as a result of a mutation causing loss of wild type adaptation (breakdown of mode of seed dispersal and loss of germination regulation). It has been proposed that the nutritive value of chickpea, especially its high tryptophan levels (1.1mg/g seed dry weight) that synthesize human brain serotonin, the feel-good hormone, may account for its domestication and the struggle to keep growing such agronomically

(a)

(b)

Figure 2.1. Picture showing: (a) desi flowered plant and dried seeds (b) kabuli flowered plant and dried seeds. Please look for differences in flower and seed colour.

complicated crop (Kareem et al., 2007). Abbo et al. (2005) proposed that the interaction between man and plants in the Near East was driven by the plants' nutritional features as well as cultural forces several years ago. The ancient conversion of chickpea into summer crop, vernalization insensitivity rather than pod indehisence or free germination makes chickpea domestication exceptional from all other crops (Abbo et al., 2003). The Fertile Crescent where chickpea was domesticated is the primary center of diversity and the Mediterranean Europe, Indian subcontinent, north-east Africa, Mexico and Chile being some of the secondary centers of diversity (van der Maesen, 1972). Upon domestication in the Middle East, chickpea spread throughout the Middle East, the Mediterranean region, India, and Ethiopia (Ladizinsky, 1975; van der Maesen, 1987).

2.1.4 Distribution

Distribution of chickpea occurs from sea level to >5000m. *Cicer arietinum* L. is found only in cultivation and the wild species such as *C. reticulatum* and *C. bijugum* occur in weedy habitats; *C. pungens* and *C. yamashitae* in mountain slopes; *C. montbretii* and *C. floribundum* on forest soils and in braod-leaf forests (ICRISAT, 2007). *Cicer reticulatum* has limited distribution, mainly found in the south eastern Turkey (Redden and Berger, 2007). Dark seeded desi type is mainly found in Ethiopia, Turkey, Iran, and India (van der Maesen, 1972). Chickpea had spread widely to Crete in the west, upper Egypt in the south, eastwards through modern Iraq to Indian sub-continent by 2800-1300 BC (Vishnu-Mittre and Savithri 1982). However, chickpea distribution continued to South and West Asia and Ethiopia for the fisrt time by 1300-500 BC (Redden and Berger, 2007). Presently desi types prevail in the Indian subcontinent, Ethiopia, Mexico, Iran whereas Kabuli types are mainly grown in Southern Europe, Northern Africa, Afghanistan, Chile and introduced to India only in the 18th century. Introduction of chickpea to the New World in the 16th century AD was by the Spanish and Portuguese (van der Maesen, 1972), however chickpea cultivation and genetic improvement in the USA, Australia and Canada are recent occurrence (Redden and Berger, 2007).

2. 2 Utilization of Chickpea

2. 2.1 Human food

Chickpea, one of the most favored legumes globally is consumed in various forms and preparations. In India, unripe chickpea seeds are eaten as raw snack whereas leaves are eaten as green vegetables (Muehlbauer and Tullu, 1997). Chickpea leaves are a good source of several minerals required by humans and nutrients levels significantly exceeded those previously reported for spinach and cabbage (Ibrikci et al., 2003). In Turkey and India, the countries with the respective highest and second highest per capita consumption globally, chickpea is consumed as besan (flour), dhal (shelled and split seeds), and whole grain in order of decreasing preference (Yadav et al., 2007). Mature chickpeas are eaten in salads, cooked in stews, ground into flour and used in different ethnic cuisines, ground and shaped into balls and fried and roasted, spliced and eaten as a snack (Muehlbauer and Tullu, 1997).

Until now, most legumes including chickpea have constantly been accompanied by species that produce carbohydrates, ie cereals in temperate zones and tubers in tropical zones (Yadav et al., 2007). However, chickpea seed, a major carbohydrate source, has been reported to have starch contents from 30 to 57% and amylose contents from 20 to 46% (Wood and Grusak, 2007). This makes chickpea a complete diet as it has appreciable levels of the limiting amino acids; methionine, lysine, leucine, isoleucine, tryptophan, threonine, valine, and phenylalanine (Wood and Grusak, 2007). Cystein, tyrosine, histidine, and argenine, all amino acids required by infants and growing children are constituents of chickpea seeds.

2.2.2 Human health benefits

Glycemic index (GI) defines amount of glucose released into the blood after food ingestion (Jenkins et al., 1981). A low GI food will release energy slowly, steadily and it is generally appropriate for everyone, especially diabetics (Salmeron et al., 1997, Lui et al., 2001), dieters and endurance athletes. Chickpeas have a low GI making them good for people with blood glucose problems (Jenkins et al., 1983). Mendosa (2009) reported a GI of 10 for chickpea compared to 100 for potato and bread. He noted that GI is about quality and not quantity of glucose and the smaller the GI value the better. Chickpea-based chapatti, a popular bread made of chickpea flour mixed with wheat in various ratios, is served as a special component of lunch or dinner (Yadav et al., 2007). It has been recently found that addition of chickpea flour to wheat

flour in chapattis increased its resistant starch significantly from 0.66% (100:0, wheat-chickpea flour ratio) to 3.22% (60:40, wheat-chickpea flour ratio) (Utrilla-Coello et al., 2007). Hydrolysis index and glycemic index, respectively, decreased from 66.3% and 65.3% (control wheat) to 30.7% and 34.6% (60:40, wheat-chickpea flour ratio). It is strongly believed that high amylose chickpea chapatti will experience increased sales and consumption, since high amylose maize bread had a 1.4% increase in bread consumption in Australia (Brown, 1996).

Chickpea is one of the most hypocholesteremic agents among food legumes, as germinated chickpea was shown to be most effective in controlling cholesterol level in rats (Geervani, 1991). Duke (1981) reported that chickpea is considered a cholesterol reducer due to their unsaturated fatty acid (primarily high in linoleic and oleic acids) and fiber content. Both the desi and kabuli type chickpea have higher oleic acid to linoleic acid (Desi: oleic 52.1, linoleic 38.0; Kabuli: oleic 50.3, linoleic 40.0). Chickpea have also been suggested to increase sperm and milk production in humans and animals, provoking menstruation and helping to treat kidney stones (Muehlbauer and Tullu, 1997). Malic and oxalic acids, glandular secretions harvested from chickpea plants have important medicinal values in treating bronchitis, catarrh, cutamenia, cholera, constipation, diarrhea, dysepepsia, flatulence, snakebite, sunstroke and warts (Duke, 1981).

2.2.3 Animal feed

Comparing eight adapted lines each of desi (Indian origin) and kabuli (Mediterranean origin) for their nutrient digestibility in swine, the digestibility coefficients for dry matter (83.1 vs 72.5%), gross energy (83.5 vs 74.8%) and crude protein (83.7 vs 79.4%) were higher for kabuli than desi type chickpea. Therefore kabuli chickpea will have greater potential as protein and energy source for use in swine rations than desi (Thacker et al., 2002). However, Salgado et al. (2001) reported that both kabuli and desi chickpea seem to be satisfactory protein and energy sources for weaned piglets. By soaking in basic medium (sodium bicarbonate solution, 0.07% pH=8.4), the nutritional value of raw and processed chickpea protein with or without cooking led to the highest food intake, nutritive utilization of protein, and weight gain in rats (Nestares et al., 1996).

Monogastric animals and human intestinal mucosa lacks α-galactosidase enzyme required for hydrolyzing α (1-6) linkages of raffinose family of oligosaccharides (RFO) hence RFO are classified as non-digestible carbohydrates (Gitzelman and Auricchio, 1965). RFO produces

flatulence upon microbial degradation in the colon. Net energy value of legume seeds which contains high amounts of α-galactosides is low since net efficiency of digestible energy utilization due to fermentation is 70% than that of glucose absorbed in the upper gut (Muller et al., 1989; Hedley, 2001). Therefore it is desirable that RFO levels in legumes be reduced through addition of microbial α-galactosidase in feed formulations or reduce seed RFO concentration by plant breeding to improve its nutritional quality.

2.3 Chickpea germplasm

2.3.1 Germplasm collection

The ICRISAT genebank contains the largest collections of 16,991 accessions of chickpea from 44 countries (Upadhyaya, 2003). Of these, 4,150 accessions were obtained from 65 collection missions in 14 countries, Afghanistan, Bangladesh, Ethopia, India, Kenya, Malawi, Morroco, Myanmar, Nepal, Pakistan, Syria, Tanzania, Turkey and Uganda. The other 12, 842 accessions of cultivated species were obtained from donations by 42 countries. According to Redden and Berger (2007), the greatest diversity of the largest gene bank for chickpea landraces (17,250 accessions) at ICRISAT is from India, Iran, Ethiopia, Afghanistan, Pakistan, Turkey, Mexico, Syria, Chile, former Soviet Union, and regions like southern Europe, northern Africa, eastern Africa, South America, North America (Table 2.1).

2.3.2 Mini core

The concept of mini core collection, a representative sample of whole collection with maximum diversity but minimum repetitiveness was introduced by Frankel (1984). Accessions from adjacent countries with similar agroclimate were grouped together in developing the core collection (Upadhyaya and Ortiz, 2001). Forty sets including one set with 165 accessions of unknown origin constituted the entire collection (16,991 accessions) held at ICRISAT genebank. Each entry was planted on a 4m-row ridge. Data on geographic distribution or origin and quatitative traits such as days to 50% flowering, days to maturity, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, tertiary branches, 100-seed weight, plant height, plant width, number of pods per plant and number of seeds per pod, seed yield were used for clustering. Standardized data (Milligan and Cooper, 1985) was subjected to hierarchical cluster alogarithm (Ward, 1963) at an R^2 (squared multiple correlation) value of 0.75 with SAS (SAS Institute, 1989). This procedure minimizes sum of squares within

(error) groups and maximizes the sum of squares among (traits) groups. Aproximately 10% of accessions were randomly selected for inclusion into core subset from each cluster. Clusters that had less than 10 accessions had at least one accession randomly selected and included in the core. Sixty-three accessions from 12 countries did not have quantitive data available and six accessions were selected randomly and included in the core. To determine whether the core subset represented the entire germplasm for each of the 13 traits, the Wilcoxon (1945) rank-sum non-parametric test was performed with the SAS NPAR1-WAY procedure (SAS, 1989). The 16, 991 entire chickpea collection produced 1, 956 chickpea core collection entries consisting of 1465 desi, 433 kabuli and 58 intermediate type. Chickpea mini core represents 1% of entire collection at a given time **a**nd captures 70% of the useful genes of the entire gene pool (Upadhyaya and Ortiz, 2001).

To develop the mini core, the 1,956 core collections were planted in the Vertisols (Kasireddipally series-Isohyperthermic Typic Pellustert) in the field in the 1999/2000 post rainy season at ICRISAT centre, Patancheru, India (Upahyaya and Oritz, 2001). Five plants were randomly selected from each entry and observations recorded on plant height, plant width, number of apical primary branches, apical secondary branches, basal primary branches, basal secondary branches, tertiary branches, number of pods per plant, seeds per pod, 100-seed weight and plant yield. Flower colour, plant colour, growth habit, seed colour, seed shape, dots on seed testa and seed testa texture; all morphological descriptors were recorded by IBPGR, ICRISAT and ICARDA (1993). Days to 50% flowering, days to maturity, flower duration (days between 50% flowering and end of flowering in 50% plants) and pod yield were recorded on plot basis. Statistical analysis was the same as that for core selections. Proportional strategy was used where approximately 10% of the accessions were randomly selected from each cluster to constitute the mini core subset. At least one accession was randomly selected from clusters which had 10 accessions. Shannon-Weaver (1949) diversity index (H) was estimated as a measure of phenotypic diversity of each trait independently for core and mini core and compared if diversity of each trait was retained. The 1,956 core subset resulted in 28 clusters which produced 211 entries from core subset as mini core. Percentage proportions of accessions retained, trait mean scores, variances, median scores, coefficient of variation and Shannon-Weaver diversity indices; all indicated that the mini core subset composition reflected the core subset and the entire collection.

Table 2.1: Chickpea accessions collected and housed at ICRISAT

Country	Number of accessions			
	Core collection*	Minicore** chickpea types		
		Desi	Kabuli	Intermediate***
Afghanistan	700	3	4	-
Chile	139	1	0	-
Ethiopia	930	13	1	-
India	6930	82	9	-
Iran	4850	39	13	-
Mexico	390	3	1	-
Pakistan	480	4	-	-
Soviet Union	133	2	4	-
Syria	220	-	2	-
Others	2398	12	10	8
Total	17,250	159	44	8

*Entire chickpea accessions at ICRISAT gene bank in 2007 (Redden and Berger, 2007).

**Mini core accessions (Upadhyaya and Ortiz, 2001) developed from 16, 991 accessions.

*** Accessions could not be classified as desi or kabuli

2.4 Chickpea seed composition

Chickpea is a good source of carbohydrates and proteins, both accounting for about 80% of seed total dry weight (Singh, 1985). Seed coat constitutes 14% of the desi seeds compared with 6% of seed coat weight of kabuli types (Saini and Knights, 1984). Chickpea and beans have slightly higher starch concentration as compared to lentils and peas, with chickpea showing a much larger variation in amylose concentration as compared to other pulse seeds (Table 2.2).

2.5 Carbohydrates

Carbohydrates have been classified into monosaccharides, disaccharides, oligosaccharides and polysaccharides (Chibbar et al., 2004). Carbohydrates are derived from simple sugars like glucose, fructose and galactose, therefore reducing or increasing any of these compounds will affect other carbohydrate constituents (Hedley, 2001). Carbohydrates in human nutrition can be classified into available (mono and disaccharides), which are enzymatically digested in the small intestine and unavailable (oligosaccharides, resistant starch, non-cellulosic polysaccharides, pectins, hemicellulose and cellulose), which are not digested in the small intestine (Chibbar et al., 2004). The latter carbohydrates are fermented by microflora in the large intestine releasing short chain fatty acids, carbon dioxide and methane gas among others (Hedley, 2001).

2.5.1 Monosaccharides

As primary source of energy, chickpea monosaccharides such as glucose, fructose, ribose, and galactose exist in their phosphorylated forms in mature seeds. They are mostly transitory intermediates in the synthesis of more complex carbohydrates (Buchanan et al., 2005). The Maillard reaction reduces monosaccharides that cause oxidative stress through free radical formation especially during seed maturity and germination, hence it has been suggested it is advantageous to a plant to have reduced monosaccharides in its seeds (Sun and Leopold, 1995). Sanchez-Mata et al. (1998) reported chickpea monosaccharide concentrations for galactose (0.05%), ribose (0.1%), fructose (0.25%), glucose (0.7%).

2.5.2 Disaccharides

Maltose (0.6%) and sucrose (1-2%) have been reported to be the most abundant free disaccharides in chickpea (Wood and Grusak, 2007). Maltose is a reducing sugar, whereas

sucrose is a non-reducing sugar. In other pulses, sucrose concentrations vary from 1.7% to 4.7% in bean, pea, lentil, faba bean and chickpea seeds, respectively (Table 2.2).

2.5.3 Oligosaccharides

Polymeric sugars of 3-20 monosaccharides are called oligosaccharides (Chibbar et al., 2004). Pulse seeds contain the highest concentrations of oligosaccharides among the crops. Sucrose is the precursor for oligosaccharide synthesis, for example, transfer of galactinol (α-1, 1-myoinositol) to the C-6 hydroxyl group of the terminal D-glucosyl of sucrose molecule forms the trisaccharide raffinose in the presence of raffinose synthase (Buchanan et al., 2005). The tetrasaccharide stachyose and the pentasaccharide verbascose are synthesized in a similar process (Peterbauer et al., 2001). Oligosaccharides are not absorbed or hydrolyzed by human digestive system but fermented by colonic bacteria to release gases or flatulence (Kozlowska et al., 2001). However, the fermented products like short chain fatty acids are reported to increase bifidobacteria population thereby improving colon health (Tomomatsu, 1994). Total α-galactosides reported are 3.0% (faba beans), 3.2% (lentils), 3.8% (beans and chickpeas) and 4.6% (Peas) (Table 2.2).

2.5.4 Polysaccharides

Polysaccharides are high molecular weight monosaccharide polymers present as storage carbohydrate (example starch) or as structural carbohydrates (e.g. cellulose) providing structural support (Wood and Grusak, 2007). Among the storage polysaccharides, chickpea is reported to synthesize and store starch and not galactomannans (Wood and Grusak, 2007).

2.6 Starch

Starch, a major source of calories in the human diet and in animal feed, is the predominant storage carbohydrate reserve in the seeds of grain legumes. The starch quantity is a major contributor to seed weight and affects the grain yield. Starch is made up of two large glucan polymers, amylose ($10^5 – 10^6$ Da) and amylopectin ($10^7 – 10^8$ Da), in which the glucose residues are linked by α-(1→4) bonds to form a chain (Figure 2.2). The glucan chain is branched by α-(1→6) bonds, which are frequent (up to 5%) in amylopectin and infrequent (<0.5%) in amylose (Figure 2.2). The ordered nature of the α-(1→6) bonds, glucan branch chain length and their

Table 2.2 Carbohydrate concentration (%) of some pulse seeds.

Constituents	Bean	Pea	Lentil	Faba bean	Chickpea
Water -Soluble carbohydrates					
Fructose	-	-	0.1	0.4	-
Sucrose	2.5	2.1	1.7	2.2	4.7
Raffinose	0.7	0.9	0.3	0.5	0.3
Ciceritol	-	-	0.7	Not detected	2.2
Stachyose	2.7	2.0	1.9	0.9	1.3
Verbascose	0.6	1.8	0.3	1.8	Trace
Total α-galactosides	3.8	4.6	3.2	3.0	3.8
Total soluble sugars	5.2	6.7	5.0	5.6	8.4
Water-Insoluble Carbohydrates					
Starch	54.0	39.0	47.4	43.0	50.4
Amylose	27.2-29.5	23.9-24.1	23.5-24.7	24.0	20.0-46.5
Resistant starch	8.2	10.1-13.3	14.7	33.0	3.4-16.4
NDF	8.9-12.8	13.2-25.6	9.7-24.1	13.0-19.5	7.5-19.2
ADF	3.5-7.2	no information	2.0-6.8	10.3-11.4	3.8-14.7
Cellulose	3.2-13.1	0.9-13.3	3.5-14.8	8.3-14.3	1.1-13.7
Hemicellulose	0.5-5.6	0.9-12.4	1.2-15.7	1.6-8.9	0.6-16.0
Lignin	0.1-3.1	0.3-2.1	Trace-2.6	0.7-2.0	Trace-7.1
TDF	11.2-27.5	16.1-21.6	11.0-21.4	17.1-23.8	8.2-24.0
SDF	8.1-10.0	4.6-6.0	1.2-4.4	6.0-8.7	3.7
IDF	9.1-11.6	11.6-16.1	8.8-13.7	8.3-15.5	7.9
NSP	6.4-20.4	no information	6.9-14.7	17.5	5.5-35.4
Total CHO	67-71	69.7-71.3	67.8-72.3	52-54	50.0-73.1

Source: Wood and Grusak, 2007; Kozlowska et al. 2001; Sika et al.1995; Chung et al. 2008; Hoover and Ratnayake, 2002; Guillon and Champ, 2002; Bello-Perez et al. 2007. Abusin et al. 2009. NDF-neutral detergent fibre, ADF-acid detergent fibre, TDF-total dietary fibre, SDF-soluble dietary fibre, IDF-insoluble dietary fibre, NSP-non-starch polysaccharides

Table 2.3 Comparison of selected extrinsic characters and carbohydrate composition of desi and kabuli chickpea whole seeds.

Extrinsic characters / Constituents	Desi		Kabuli	
	No. of cultivars	Mean	No. of cultivars	Mean
Extrinsic Characters				
Thousand seed weight (g)	5	13.9	1	21.9
Mean seed diameter (mm)	5	6.6	1	7.7
Intrinsic Characters				
Water Soluble Carbohydrates (% dry weight)				
Sucrose	1	1.5	3	2.0
Raffinose	1	6.8	3	1.7
Ciceritol	10	2.0	-	-
Stachyose	1	21.1	3	2.6
Verbascose	1	0.6	1	0.0
Water Insoluble Carbohydrates (% dry weight)				
Starch	1	42.9	2	46.3
Starch granule length (µm)	5	18.3	1	19.9
Starch granule width (µm)	5	12.2	1	14.4
Amylose	5	31.1	1	30.4
Resistant starch	1	6.4	1	3.9
Total CHO	5	64.7	1	66.9
Others (% dry weight)				
Ash	5	2.9	1	3.1
Fibres	5	10.3	1	4.6
Fat	5	3.6	1	5.5

Source : Singh et al. 2004; Salgado et al. 2001; Attia et al. 1994; Chung et al. 2008

Table 2.4 Comparison of protein and amino acid composition of desi and kabuli chickpea whole seeds.

Constituents	Desi		Kabuli	
	No. of cultivars	Mean	No. of cultivars	Mean
Total protein (%)	8	22.2	7	23.2
Amino acids (g/kg)				
Alanine	1	8.9	1	11.4
Arginine	1	22.4	1	23.2
Aspartic acid	1	23.1	1	29.5
Glycine	1	7.9	1	11.2
Glutamic acid	1	43.6	1	54.5
Histidine	1	6.9	1	8.9
Isoleucine	1	9.1	1	11.4
Leucine	1	16.3	1	20.6
Lysine	1	12.4	1	17.0
Methionine	1	2.6	1	3.8
Phenyalanine	1	13.2	1	16.8
Serine	1	11.8	1	14.2
Threonine	1	8.4	1	11.6
Tyrosine	1	6.7	1	8.5
Valine	1	9.4	1	11.5
Tryptophan	3	12.0	4	14.0

Jambunathan and Singh, 1980; Viveros et al. 2001; Ribeiro and Melo, 1990

Table 2.5 Comparison of mineral composition and concentration (mg $100g^{-1}$) desi and kabuli chickpea whole seeds.

Minerals	Desi		Kabuli	
	No. of cultivars	Mean	No. of cultivars	Mean
Cu	16	1.3	21	1.2
Fe	16	4.5	21	4.5
Zn	16	3.6	21	3.5
Mn	16	1.7	21	1.6
Ca	16	210	21	154
Mg	16	128	21	122
Na	16	23	21	21
K	16	878	21	926
P	8	380	3	235

Ibanez et al. 1998; Thacker et al. 2002; Ribeiro and Melo, 1990

branching pattern, form clusters of branches within an amylopectin molecule. Three main types of glucan chains, short A-chains, intermediate B-chains and the long inter-cluster C-chains (Figure 2.3). provide the unique and highly-ordered structure of the amylopectin molecule, which is essential to the formation of the starch granule. Both polymers are packed into water insoluble granules to form a three-dimensional semi-crystalline structure (Figure 2.4). In a normal starch granule, amylose and amylopectin are present in a ratio of 1:3, along with small amounts of lipids, protein phosphate. Both the relative proportion of amylose to amylopectin and their molecular structure which vary with the botanical species have significant influence on starch properties and end-use of starch and grains.

2.6.1 Starch granule structure and organization

The size and shape of starch granules also varies with the plant species adding to the natural diversity of starches. The size and shape of starch granules also influence the end use of starch (Lindeboom et al., 2004). Singh et al. (2004) reported chickpea starch as large-oval to small-spherical shaped granules with a smooth surface and no fissures. Chickpea starch granule length and width ranged between 17-20.1 and 11-14.4 µm, respectively (Singh et al., 2004). Hoover and Ratnayake (2002) reported a range of 22.0-22.4 and 18.5-18.8 µm, respectively for chickpea granular length and width. The granule size of pea, a pulse crop has been reported to be 5-38 µm and monomodal in distribution, similar to that of waxy maize and B-granule potato starch (Fredriksson et al., 1998). Amylopectin structure is composed of A, B and C chains (Figure 2.3; Hizukuri, 1986). A chain binds to B chains only. B binds to other B or C chain, which has a reducing end. A chains are shortest, while C chains are longest, and B1-B3 chains have a degree of polymerization in between the A and C chains. Hanashiro et al. (1996) suggested the classification of amylopectin chain length into four fractions: A chain fractions (DP6-12), B1 fractions (DP13-24), B2 fractions (DP25-36) and B3 fractions (DP≥37). Amylopectin chain lengths from different wheat sources had fractions A, B1, B2, and B3 ranging from DP12-16, DP20-24, DP42-48 and DP69-75, respectively (Yasui et al., 2005). Shibanuma et al. (1994) reported amylopectin chain length fractions of A, B1, B2, and B3 as DP10-13, DP20-23, DP41-43 and DP68-71, respectively, in bread wheat. Amylopectin chain length distribution profiles are plant species specific, and these could potentially be used as fingerprints to identify plants from which the starch was extracted (Koizumi et al., 1991).

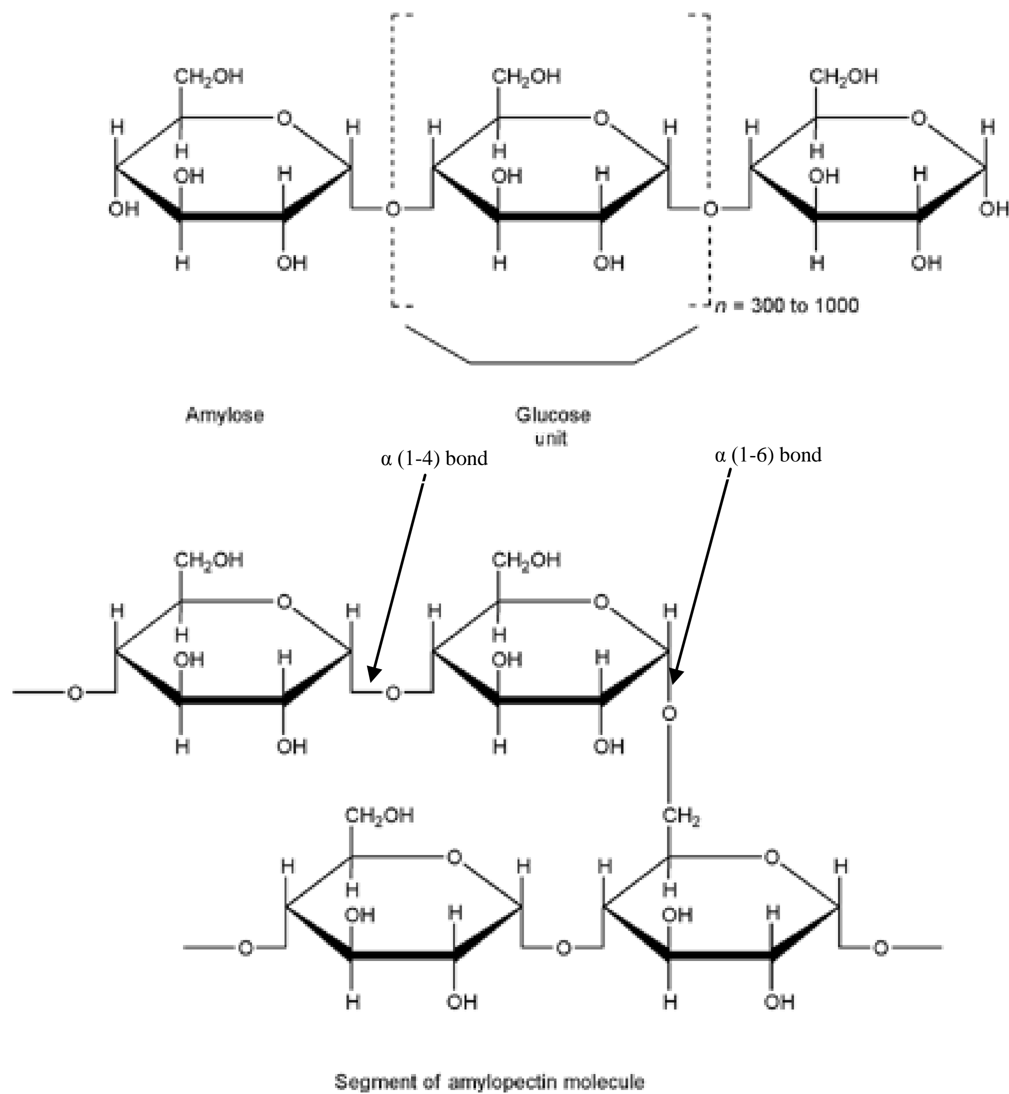

Figure 2.2 Amylose (top) and amylopectin (bottom) linear structures. Adapted from Google Images, 2009.

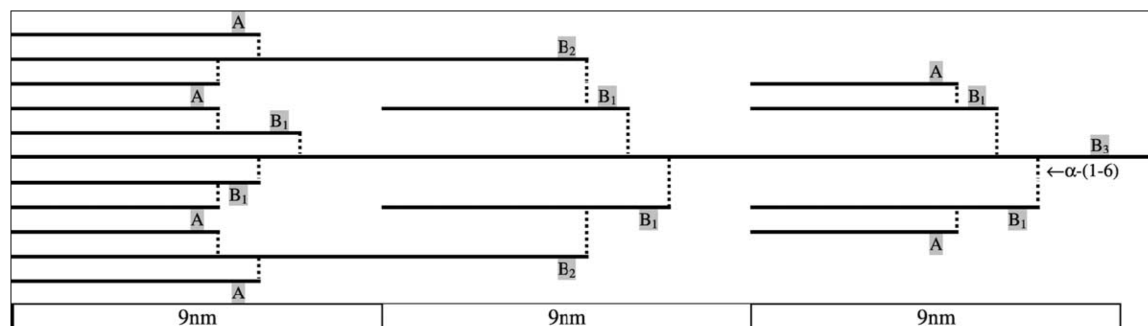

Figure 2.3. Branching pattern of amylopectin schematically representing α-(1-4) chains (A, B1-B3) joined by α-(1-6) linkage branch points. Adapted from Hizukuri (1986).

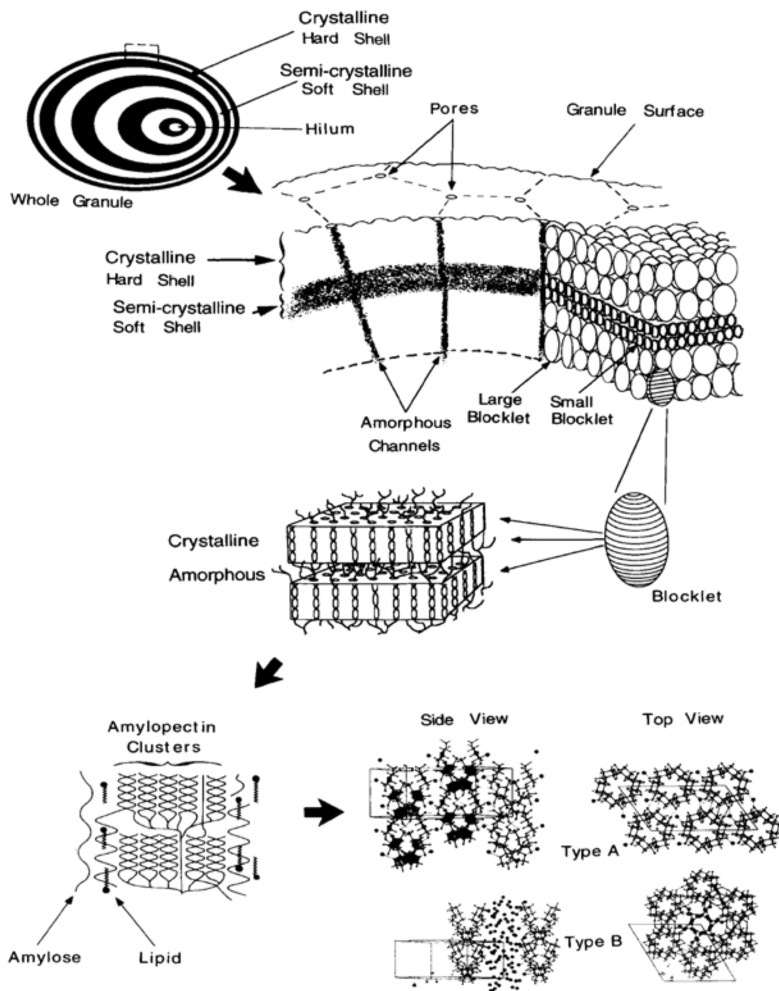

Figure 2.4. Overview of the organization of starch granule structure. From lowest level (top) of hard crystalline and soft amorphous/semicrystalline shells of the granule to the highest level (bottom) of crystal structure types A and B. Adapted from Gallant et al. 1997.

2.6.2 Starch Biosynthesis

Starch is the major storage carbohydrate of many seeds especially of grains consumed by humans, yet starch structure and biosynthesis are not completely understood (Zeeman et al., 2010). Starch is synthesized and stored in the plastids, temporarily in the chloroplast of leaves and in a long-term in the amyloplasts of roots, tubers and seeds. Starch synthesis protects plants from osmotic disruption, without which sucrose would flood the plastids, absorbing water from the cytosol and causing plastids to swell and burst (Michalska et al., 2009).

Studies of starch mutants have produced a general consensus that ADP-glucose pyrophosphorylase (AGPase), soluble starch synthases (SS), starch branching enzymes (SBE), starch debranching enzymes (DBE; pullulanase; isoamylase) and possibly a disproportionating enzyme (D-enzyme) catalyze the final steps leading to amylopectin synthesis. Several of the enzymes exist in different isoforms, some of which vary in their sub-cellular distribution, enzyme specificity, temporal activity and interactions with other enzymes, making the starch synthesis pathway very complex. A minimal subset of 14 conserved starch biosynthetic enzymes (two AGPases, five SS, three SBE and four DBE) is homologous in all plant species studied to-date (Morell and Myers, 2005). Some of these isoforms have specific functions in starch biosynthesis.

ADPglucose pyrophosphorylase is a key regulatory enzyme in starch biosynthesis. It is allosterically regulated by both inorganic phosphate (an inhibitor) and 3-phosphoglycerate (an activator) (Preiss and Sivak, 1996). The priming mechanism for starch glucan polymerization in plants is unclear, but likely involves short-chain maltodextrins that are extended and branched by the action of GBSS1, SBE1 and SS1 to form a molecule with amylopectin-like backbone. The primary glucan polymer is further polymerized through cycles of chain extensions and branching catalyzed mainly by SSI and SBEII. Starch synthases catalyze the transfer of a glucosyl moiety from ADP-glucose to the non-reducing end of an α(1-4) glucan primer. Plants produce several SS isoforms which include GBSSI, SSI, SSII, SSIII and SSIV, of which all but SSIV have been shown to have distinct roles in determining starch composition and structure. Opinions still differ as to whether ADP-Glc is added to the reducing end without a primer (Mukerjea and Robyt, 2005a; Mukerjea and Robyt, 2005b; Mukerjea et al., 2002) or nonreducing end with a primer (Denyer et al., 2001; de Fekete et al., 1960). Besides the core enzymes (Morell and Myers, 2005), additional enzymes such as starch phosphorylases, disproportionating enzymes and

glucan water kinases also play important roles in starch biosynthesis. Recent studies have also shown post-translational modifications of some core starch biosynthetic enzymes (Tetlow et al., 2004), introducing a new level of complexity for modification of starch structure in planta. Recently, Tetlow et al. (2008) reported that 260kDa protein-protein complexes (SS-SBEII complex) in wheat amyloplasts revealed functional interactions among starch biosynthetic enzymes. It was concluded that the 260kDa SS-SBEII protein complexes were formed 10-15d after pollination and prior to that stage, SSI, SSII and SBEII forms were detectable only in monomeric forms. Amylose and extra-long unit chains of amylopectin are primarily synthesized by granule-bound starch synthase I, a class of the starch synthases (Hanashiro et al., 2008).

2.7.1 Seed starch isolation methods

Legume grain starch isolation is difficult because of insoluble flocculent protein (~0.3-0.4%) and fine fibre, which decrease sedimentation and co-settles with starch giving a brownish deposit (Hoover and Sosulski, 1990). Lineback and Ke (1975) isolated chickpea and horse bean starch by steeping flour (1000 g, 3L) overnight. Residual pulp was re-washed and rescreened with distilled water and 60-mesh sieve, respectively. Starch was centrifuged at 2000 x g for 20min, supernatant decanted, brown upper layer removed with spatula and residual starch was air-dried at room temperature.

Demeke et al. (1999) and Zhao and Sharp (1996) steeped cut wheat seeds in sterile distilled water over night at 4°C. Water was decanted and seeds were ground into slurry with a pestle. Slurry was layered over cesium chloride (80% w/v) in 2mL microfuge tube and centrifuged at 13000 x g for 5min. This step was repeated twice to remove any adhering non-starch molecules. Starch granules were washed twice with tris-HCl buffer (pH 6.8) and granules air-dried over night before used for amylose determination. Miao et al. (2009) soaked overnight at 20°C, chickpea seeds in excess of distilled water containing 0.2% sodium hydrogen sulphite. Testa of seeds was removed manually, decorticated grains ground in laboratory blender, slurry was filtered through 100-mesh sieves and centrifuged at 3000 x g for 20min. Sediment was washed thoroughly with distilled water and washing repeated until starch was free of colour. Starch was oven dried at 40°C for 12 hours.

To isolate various components of chickpea seeds, dehulled chickpea seeds were milled with GM 280/S-D (Conduk Werk, Hannau, Wolfgang, Germany) (Emami et al., 2006). Sodium hydroxide (10M NaOH) was added to 5% (w/w) slurry bringing pH to 9.0 and stirred at 20°C for

1h. Slurry was left over night, reducing pH to 7.5-8.0, pH adjusted to 9.0 and stirred at 20°C for one hour. Slurry was transferred to feed tank of hydrocyclone system and processed for starch, protein, total diatery fibre, fibre, fat and ash employing centrifugation. Precipitates were washed with distilled water, pH adjusted to 4.3, centrifuged, freeze dried and ground in Willey Mill with mesh of 2mm.

2.7.2 Total starch determination methods

Quantification of starch, the most important component of economically important grains, is very relevant to both research (proximate composition, purity check) as well as commercial (nutrition labeling, pricing and selection) applications (Vasanthan, 2001). Polarimetric (Mitchell, 1990), acid and enzymatic hydrolysis procedures are the major starch determination methods employed (Anon, 1987). In polarimmetric methods, samples are treated with HCl, stirring in a bath of boiling water, and in 4% sodium phosphotungstate and saccharimeter readings are taken at different time intervals. The polarimetric method is of little value as it underestimates total starch content (McCleary et al., 1994).

Under acid hydrolysis, a sample is treated with trifluoro acetic acid, followed by heating, treatment with sodium acetate (pH5.0) and glucose oxidase peroxidase (GOPOD) reagent (McCleary et al., 1994). The acid hydrolysis procedure complicates results because it can lead to the release of glucose from other polysaccharides such as *beta*-glucan in oat bran, amorphous cellulose and *beta*-glucan in chicken feed pellets. Acid hydrolysis is effectively applicable only to purified starch samples and therefore has limited applications.

In the AACC approved methods (1985 and 1990), for starch hydrolysis a sample is suspended in water and gelatinized by autoclaving and treated with amyloglucosidase (55°C) to convert starch to glucose. McCleary et al. (1994) reported that this enzymatic method as well as acid hydrolysis method underestimated total starch in samples containing resistant starches, high amylose maize and cross-linked starches. In McCleary et al. (1994) modified method; starch is converted to soluble fragments by treatments with thermostable α-amylase at 100°C followed by pullulanase/*beta*-amylase at 50 ^{o}C. Starch dextrins are hydrolysed quantitatively to glucose with amyloglucosidase. Free glucose is then quantified as total starch after reaction with glucose oxidase/ peroxidase reagent (GOPOD) at absorbance of 510 nm on a spectrophotometer. Following its accuracy and reproducibility of starch in an interlaboratory evaluations, the

American Association of Cereal Chemists and Royal Australian Chemical Institute have adopted McCleary et al. (1994) modified method as a standard procedure.

2.7.3 Amylose determination methods

Measurement of amylose concentration is an important quality parameter because some properties of starches that determine their suitability for particular end-uses are dependent on their amylose/amylopectin ratios (Gibson et al., 1997). Comparing differential scanning calorimetry, high-performance size-exclusion chromatography (HPSEC) and iodine-binding methods, double iodine binding procedure at 620nm and 510nm wave lengths increased precision in amylose concentration (Zhu, 2008). Iodine binds to amylose causing conformational change from flexible coil to helix yielding blue colour (Banks and Greenwood, 1975). However, amylopectin binds weakly to iodine to form reddish brown colour probably due to its short chain lengths (DP≤20) (Wang et al. 1998). In determining amylose by iodine inclusion complexes using colourimetric methods, amylopectin-iodine complexes also form which may absorb at similar wavelengths to amylose-iodine complexes (Takeda et al., 1987) leading to overestimation of amylose. Moreover, amylose calibration curves do not account for absorption by amylopectin-iodine complexes and commercial sources of amylose for amylose calibration curves vary in their purity and iodine binding capacity (Welsh and Blakeney, 1992). Wavelength of maximum absorbance of amylose-iodine complexes increases with increasing degrees of amylose polymerization (Banks and Greenwood, 1975). Iodine binding should be used as a guide to determine the nature of the polymer and not as classification method (Yu et al., 1996).

Differential scanning calorimetry procedure for amylose determination is not influenced by the presence of amylopectin or indigenous lipids (Mestres et al., 1996). Moreover near-infrared transmittance spectroscopy (NITS) (Villareal et al., 1994) and differential scanning calorimetry (DSC) (Mestres et al., 1996) are rapid procedures for amylose determination. Both NITS and DSC eliminate much wet chemistry but require reference method for calibration. Other drawbacks of NITS and DSC have been lower penetration of light in whole grains giving less accurate results (Kays et al., 2005) and several mathematical and regression models required for calibration (Delwiche et al., 1995).

In another method a lectin concavalin A was used to precipitate amylopectin from a starch solution and determines the residual amylose by converting it in to glucose (Gibson et al.,

1997). This method has several benefits such as applicability to starch samples from various sources with full range of amylose concentrations, avoids empirical corrections necessary in methods based on amylose-iodine complex formations and does not need standard curves with varying amylose: amylopectin concentration (Gibson et al., 1997). However, a major limitation is the inability to precipitate all of the amylopectin thus giving an apparent higher amylose concentration (Demeke et al., 1999). A modified HPSEC method which uses starch debranched with isoamylase was fractionated on a size exclusion column and eluant monitored by a refractive index detector. In this method debranched amylopectin and amylose are separated on size and the integration of peak area is used to calculate amylose to amylopectin ratio (Demeke et al., 1999).

2.7.4 Amylopectin chain length determination methods

High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is the most selective high performance/pressure liquid chromatography (HPLC) method available for separating monosaccharides, oligosaccharides and homopolymer series of oligosaccharides (Hardy and Townsend, 1988). However, HPAEC provides individual DP information but only for chains with DP of a maximum of about 80 (Broberg et al., 2000). O'Shea and Morell (1996) described a novel method in which debranched starch molecules were tagged with 8-amino-1,3,6-pyrenetrisulfonic acid (APTS), electrophoresed on slab gel and separated fragments were detected using a fluorescent detector using a DNA sequence apparatus. This method could detect >80 dp from a 15 ng starch sample. This technology was further improved by using flourophore-assisted capillary electrophoresis (FACE) (Morell et al., 1998). This method has several advantages in resolution (DP1 to 100) and sensitivity over previously used methods as it provides capacity for facile analysis of oligosaccharide population on either molar or mass basis. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has also been used to determine amylopectin chain length distribution, but it has been found to be less reproducible than high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) method (Broberg, et al., 2000). Yao et al. (2005) concluded in a study to describe chain length distribution of debranched starch that data sets from high-performance size-exclusion chromatography (HPSEC) may be transformed to allow a similar presentation as for that obtained by FACE.

2.8.1 Resistant starch (RS)

Englyst et al. (1982) for the first time, used the term 'resistant starch' to describe a small fraction of starch that was resistant to *in vitro* hydrolysis by treatment with excessive quantitities of α-amylase and pullulanase treatment. The definition of resistant starch has been further refined to include all starch and starch degradation products that resist small intestinal digestion but get fermented in the colon of humans (Topping and Clifton, 2001). Four classes of RS are recognized (Englyst et al., 1982; Englyst et al., 1992b; Brown et al., 1995) based essentially on the mechanisms for delivering resistant starch: RS I, physically inaccessible starch, as in partially milled grains or seeds; RS II, starch granules that resist digestion, as in raw potato and green banana; RS III, retrograded starch, eg. cooked and cooled potato and bread; RS IV, chemically modified starch, eg. esterified, cross-bonded starch and processed food. Most common form of resistant starch in diet is retrograded starch (RS III) because it forms as a result of food processing (Soral-Smietana et al., 1998). Starch cooked in water beyond gelatinization temperature and cooled retrogrades. There is a strong positive correlation between amylose content and level of RS III in cereals (Hedley. 2001), however, legume starch have an amylose content ranging from 30-70%, which is high compared with cereal and tuber starches (Soral-Smietana and Dziuba, 1995).

Anaerobic bacterial fermentation of starch in large intestine produces short chain fatty acids such as butyric, propionic and acetic acids (Topping and Clifton, 2001). They further observed butyrate is the most important as it is a very important source of energy for colonocyte (Silvester et al., 1995), activates human colon cell proliferation (Mortensen and Clausen, 1996), suppress proliferation and differentiation of tumor cells (Hylla et al., 1998). Resistant starch is therefore a prebiotic as it helps grow beneficial bacteria in the large intestine improving colon and intestine health. Resistant starch-rich foods also have low glycemic index and maintains normal levels of blood glucose, insulin and cholesterol in humans (Liljeberg and Bjorck, 1994; Pereira et al., 2002; Lui et al., 2001).

Table 2.6: Classification of starch, its structure and physiological properties (Englyst et al., 1992b).

Starch Fraction	Digestion time line (*in vitro*)/ place	Examples	Amount (g/100g dry matter)	Strucutre	Main physiological property
Rapidly Digestible Starch (RDS)	Within 20 min, mouth, small intestine	Freshly cooked food	Boiled hot potato: 65	Mainly amorphous	Rapid source of glucose/energy: Leads to diabetes, obesity, cardiovascular disease
Slowly Digestible Starch (SDS)	20-120 min, small intestine	Native waxy maize starch, millet, legumes	Boiled millet: 28	Amorphous /crystalline	Slowly and sustained source of energy and sustained blood glucose
Resistant Starch (RS) Types I-IV	>120 min; not in small, mainly in colon	Raw potato, stale bread	Raw potato starch: 75	Mainly crystalline	Effects on gut health (eg. Prebiotic, fermentation to butyrate with hypothesized anti-carcinogenic effect)

2.8.2 Resistant starch measurement

The growing interest in RS has increased interest to develop a robust *in vitro* method to determine RS content in food and food products. However, the physiological definition of RS complicates the precise determination of RS as it not only includes RS fraction but also some digestible fractions (Champ et al., 2003). In addition, starch digestion in the human digestive system is affected by starch structure and the functional and physiological environment during digestion. These factors vary from one individual to other, therefore for food and food product characterization it is preferable to use an *in vitro* method for RS determination. Nevertheless, for practical application the *in vitro* assay results needed to be validated using in vivo assays done with a large number of human subjects. The main methods for RS *in vitro* assay have been recently reviewed (Champ et al., 2003). All the methods include digestion of starch with appropriate hydrolytic enzymes and separating the starch and its hydrolytic products and measuring the glucose released at specific time intervals. Several methods based on this basic principle have been reported to measure RS. Some of the modifications include changes in enzyme concentrations (Saura-Calixto et al., 1993), types of enzymes used (Muir and O'Dea, 1992; Champ 1992; Sharma et al., 2010), sample pre-treatment (Muir and O'Dea, 1992; Goni et al., 1996), pH of incubation (Champ, 1992; Saura-Calixto et al., 1993) and sample size (Champ, 1992) all of which impact RS levels in a sample.

McCleary and Monaghan (2002) assessed effects of concentrations of pancreatic α-amylase, pH of incubation, importance of maltose inhibition of α-amylase, need for amyloglucosidase inclusion, effects of shaking and stirring on determined values and problems in recovering and analyzing RS-containing pellet. The aim was to create condition that will mimic *in vivo* conditions and yield values that are physiologically significant. Pre-treatemnt of samples with pepsin (as was done by Muir and O'Dea, 1992; Goni et al., 1996) had no effect on the RS values obtained. Alcohol precipitation and washing with alcohol ensured reproducibility of RS values compared with Goni et al., 1996. The McCleary and Monaghan (2002) method has been accepted by the AOAC (2002.02) and the results are very close to those obtained in human *in vivo* assays with ileostomy patients (Champ et al., 2003).

2.8.3 Factors affecting amount of rate of starch hydrolysis and RS

Botanical sources, physical form, pre-consumption food processing, extent of chewing and intestinal transit time have been reported to affect the extent and rate of starch digestion by amylolytic enzymes (Englyst et al., 1992a). Starch crystallinity affects starch digestibility: for instance, A-type starch (eg. wheat) is digested without gelatinization whereas B-type starch (eg. firm banana or potato) is poorly digested until gelatinized (Englyst and Cummings, 1990). Starch granule size of ≤10 µm as occur in rice was reported to have higher rate of hydrolysis than starch granules of size >40µm as found in potatoes (Tatsumi et al., 2007). Grinding of rice grains decreased RS content in raw rice whereas it did not increase RS content in roasted chickpea. By cooking, RS decreased from 0.161g/g dw to 0.028 and 0.247g/g dw to 0.032g/g dw in oats and raw bananas, respectively. Storing boiled potato overnight at 4°C increased RS by 2.8 fold (Muir and O'Dea, 1992). Chewing on the other hand decreased amount of RS in foods.

2.8.4 Relationship between starch composition and structure with RS

Amylose concentration is one of the single most important factor affecting gelatinization and retrogradation properties of starch (Yasui et al., 2005). After gelatinization amylose reassociates and retrogrades quicker than amylopectin in non-waxy starch. Amylose molecular weight and chain length has also been associated with starch retrogradation (Takeda et al., 1983). Amylopectin short chains with DP 6-9 inhibit retrogradation in maize whereas longer side-chains accelerate starch retrogradation (Shi and Seib, 1992). However, bread wheat with high amylose starch has more side-chains with DP 6-10 and fewer side-chains with DP 11-25 than normal starch (Yamamori et al., 2000; Yasui et al., 2005). Waxy starches have lower proportions of DP 6-12 side-chains and higher proportions of DP>35 side-chains compared with non-waxy wheat starches (Sasaki et al., 2002; Yasui et al., 2005). Amylopectin chain length and amylose concentration of cereals are affected by environment and endosperm mutation (Inouchi et al., 2000). To study the effect of environmental temperature on distribution of amylopectin unit chains in rice, Inouchi et al. (2000) reported an increased amounts of short chains and decreased amount of long chains in rice plants grown at 25 ^{o}C compared to amylopectin obtained from rice plants grown at 30 ^{o}C. High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) revealed significantly increased 6 and 11-13 DP amylopectin chains of rice grown at 25°C and significantly decreased 22-24 and 29 DP

amylopectin chain lengths of rice at 30°C (Inouchi et al. 2000). Environmental temperature between 5 and 10 days after pollination strongly influenced structural characteristics of rice endosperm starches. Large proportions of amylopectin short side-chains with DP 6-10 cause low density of the granule structure, hence it degrades at a higher rate (Inouchi et al., 2000). Genkina et al. (2009) explained that short side-chains of amylopectin in a cluster within the semi crystalline ring affect the whole supramolecular granule organization and promote a more favorable penetration of water molecules and hydrated proteins within the starch granule thereby decreasing the resistance of starch to hydrolytic action.

Rice mutants RS7954, RS25 and RS26 have similar amylose contents but both RS25 and 26 showed higher RS concentration than RS7954 (Shu et al., 2007). Analysis of amylopectin structure revealed that rice mutants high in resistant starch had significantly increased levels of short chains with DP≤12, decreased intermediate chain contents with size 13≤DP≤36 and long chains with DP ≥37. Fraction A positively correlated with RS while fraction B2 negatively correlated with RS. They concluded that increased or decreased RS in rice is dependent on amylose content as well as amylopectin structure (Shu et al., 2007).

2. 9 Genotype by environment interaction and chickpea chemical composition

Genotype-environment (g x e) interactions have always been a major concern to plant breeders but its effect on grain quality has been less studied (Basford and Cooper, 1998). Basford and Cooper (1998) defined genotype x environment interaction in terms of biological or statistical concepts. Biologically, g x e interaction occurs when contributions or expression of genes regulating a trait differ among environments. Statistically, g x e interaction is significant if different pattern of response is detected among genotypes grown across environments. Falconer and Mackay (1996) defined g x e interaction as instance where "specific difference of environment may have greater effect on some genotypes than on others, or there may be a change in the order of merit of a series of genotypes when measured under different environments". Genotype-environment interaction occurs when "different genotypes respond differently to environmental changes" (Roy, 2000).

Expected mean squares from ANOVA for g x e interaction takes a form dependent of model (random, fixed or mixed) used. For instance, in a random model, both genotypes and environment used for the experiment are random for the populations whereas both are fixed in

case of fixed model (Roy, 2000). However, genotypes are fixed samples and environment random or vice versa in case of a mixed model. According to Roy (2000), "significant mean square genotype x environment interaction (MS g x e) indicates the presence of genotype x environment interaction. Significant g x e interaction means genotypes should be further tested over the selected environment. Roy (2000) stressed "a worker should not stop carrying out further analysis of g x e even if interaction mean square is not significant as one should be interested in studying the underlying stability structure of individual genotype".

Environments in g x e experiments are either micro-environment or macro-environment (Roy, 2000). Microenvironments are heterogeneities within a single plot, or even single plant, uncontrolled and attributed to error variation in statistical analysis. Macroenvironment is climatic, edaphic, or management conditions (day length, temperature, humidity, soil types, rainfall, planting dates and densities and different nutrient levels).

Basford and Cooper (1998) noted that g x e interactions complicate selection for broad adaptation rather than specific adaptation and their effect must be accounted for in selecting trait performance within selected environments. Genotypes response to environment is multivariate where stability models, concepts and measurements transform this multivariate system to univariate structure. Understanding the adaptations associated with g x e interactions requires critical knowledge of the concept of repeatability (Basford and Cooper, 1998). It is critical to define environmental aspects responsible for g x e interactions and hence the production constraints instead of focusing on search for broad and/or specific adaptation to random g x e interactions. For instance, combinations of genetic variation for resistance to rust, variation among environments for its incidence, and level of infection by the pathogen give rise to g x e interaction for economically important traits like yield and quality. The best strategy for removing the g x e interaction is to select for resistance to rust and incorporate the sources of resistance into adapted cultivars (Basford and Cooper, 1998). Variations in genetic regulations of growth and development and environmental variation in timing of stress events can cause large scale g x e interaction for yield and quality. Stratification of breeding lines into different maturity groups is the best strategy to eliminate g x e interactions. Lack of information (both time and resource wise) on detailed characterization of environments and influences of biotic and abiotic stresses on plant adaptation are some undefined causes of g x e interactions (Eisemann, et al., 1990).

Genotype-environment interaction has been partitioned into those contributing to change in rank of genotypes (cross-over interaction) and those that do not (non-cross over interaction) (DeLacy et al., 1996). Non repeatable interactions are a source of error to be factored into extensive testing of lines and broad adaptation selections whilst repeatable interactions are exploited for positive specific adaptation.

Economic implications of g x e interactions from statistical perspective have been influenced by Type I, II, and III errors where Type I and II errors refer to false rejection and false acceptance of the null hypothesis, respectively. Type III error refers to getting the rank order wrong (Basford and Cooper, 1998). Berger et al. (2007) noted that with large g x e interaction effect, genetic advancement is complicated hence g x e interaction in this situation cannot be ignored. However, further investigations need to be done to restructure the program to minimize interaction effect or exploited to develop cultivars of specific adaptation. Genotype-environment interaction analysis should be considered at the start of the investigation rather than the end, as such genotypes and environment should be well characterized to ascertain the underlying cause of g x e interaction (Berger et al., 2007).

Very little has been published on the influence of genotype x environment interaction on chickpea chemical components. Ereifej et al. (2001) reported that concentrations of chickpea seed protein, fat, fibre, ash, glucose, fructose, P, Ca, Mg, Na, and Cu were significantly affected by growing season. Cultivar x growing season interaction significantly influenced all traits except seed starch and seed sodium concentrations. Al-Karaki and Ereifej (1997) observed that variations in protein, lipids, sucrose and starch in peas were caused by the environment.

Environmental temperature influences the structure of amylopectin, hence starch content and quality. Bread wheat grown at higher temperatures had lower ratio of side-chains with DP6-12 and higher ratio of side chains with DP13-34 and DP≥35. Thus at higher tempe ratures proportions of side chains with DP6-12 decreased by 2.7-5.4%, and DP13-34 and DP≥35 increased by 1.7-3.9% and 1.1-2.2%, respectively (Matsui et al. 2003).

Genotype and environment studies on chickpea since 1971 have been compiled (Table 2.7). However, out of the 34 references, only four (Yadavendra and Dixit, 1987; Ereifej et al., 2001 and Sood et al., 2001) reported on g x e effect on protein. All the 34 references focused extensively on the effect of g x e on yield and its components.

2.10 Repeatability and heritability

Repeatability has been defined simply as the consistency or correlation between repeated measurements of the same individual (Falconer and Mackay, 1996). In other words, if a trait is repeatable then it is heritable and selectable (Boake, 1989) since its performance remains consistent over time and environment. A serious obstacle of genetic factors in evolution has been the difficulty in measuring heritability of natural populations (Boake, 1989). Useful estimates of heritability can rarely be made in field studies since statistical properties of variance components are not well understood, however, repeated measures of individual traits can easily be made (Boag, 1983 and Findlay and Cooke, 1983).

Use of variance components from a two-way ANOVA to estimate multi-environment genetic correlations (Yamada, 1962) is known to be highly biased (Fernando et al., 1984). High repeatability may accompany high heritability implying environmental variation is low and most of the genetic variation is additive in nature. However, excessively higher repeatability over heritability may be caused by high environmental variations, strong past selection or major contributions by non-additive variance such as dominance effects (Boake, 1989).
Repeatability can be used to:

- showing how much is to be gained by repetition of measurements
- setting upper limits to heritability in both broad (Vg/Vp) and narrow (Va/Vp) sense, ie. heritability cannot be greater than repeatability, however, Dohm (2002) reported that repeatability may not always set upper limits to heritability.
- predicting future performance from past records

Assumptions of repeatability are: variances of different measurements are equal and different measurements reflect genetically the same character.

Repeatability is a powerful tool for population geneticists, however it is reported that there are errors in its calculation (Lessells and Boag, 1987). For instance, a number of authors have equated Falconer's "variance" with "mean square" and miscalculated repeatability. In their analysis, they cautioned scientists not to publish or use published repeatability values unaccompanied by associated F ratios.

Repeatability, r_{approx} can be calculated as:

$\tilde{n} = (df1 + df2 + 1)/(df1 + 1)$ (1)

$r_{approx} = (F-1)/(F-1 + \tilde{n})$ (2)

where df1 is numerator or trait degree of freedom

df2, the denominator or error degree of freedom

F, F ratio value of the trait

ñ, mean group size

Reason for r_{approx} being an approximation value is that ñ has been used in place of no, the sample size.

In evaluating evolutionary role of repeatability on mating behavior, Boake (1989) defined repeatability as:

$r = Vg + Ve/ Vt$

where Vg is genotypic variance, Ve is general environmental variance, Vt is total phenotypic variance.

Repeatability, computed as a ratio can be low for two reasons (Boake, 1989). First, similarity of individual genotypes leads to relatively small numerator. This similarity could be due to genetic or environmental causes. Secondly, environmental influences can cause relatively large denominator and hence low repeatability. Factors such as temperature, soil moisture and experimental errors could affect repeatability estimates.

Heritability (in narrow sense) is defined as:

$h^2 = Va/Vt$

where Va is additive genetic variance and Vt is total phenotypic variance. Vt may be greater than Va if non-additive effects such as dominance influence a character. Thus low repeatability cannot accompany high heritability or low repeatability puts a low ceiling on heritability (Boake, 1989).

Heritability of clonal replicates or full-sibs grown in multi-environments can be estimated from mean square values of ANOVA table as the ratio of genotype-environment interaction variance to the total phenotypic variance (Schneiner and Lyman, 1989; Becker, 1984). Thus

Table 2.7 Chickpea g x e studies on agronomic traits published since 1971 - organized by publication year and methodology employed to analyse G x E interaction.

Reference	Genotypes	Traits studied	Method employed
Chandra et al. 1971	40	Grain yield	4
Tomer et al. 1973	10	Grain yield, seed weight, seed colour	1,2
Ramanujam and Gupta, 1974	35	Grain yield, seed weight	1,2
Mehra et al. 1980	11	Grain yield and components	1,2,4
Jain et al. 1984	32	Grain yield and components	1,2
Yadavendra and Dixit, 1987	3,9,11	Grain yield and components, Seed protein	1,2
Khan et al. 1988	14	Grain yield and components	1,2
Malhotra and Singh, 1991	23	Grain yield, seed weight	4,7
Singh et al. 1991	16	Grain yield and components	1,2
Singh and Singh, 1991	66	Grain yield, seed weight	1,2
Katiyar et al. 1992	20	Grain yield, seed weight	1,2
Singh et al. 1993	20	Grain yield and components	1,2
Singh and Kumar, 1994	24	Grain yield and components	1,2
Singh et al. 1995	44	Grain yield and components	1,2
Kumar et al. 1996	16	Grain yield, seed weight	1,2
Banik et al. 1997	9	Grain yield and components	1,2,4
Kumar et al. 1998	16	Grain yield and components	1,3,4
Aher et al. 1998	27	Grain yield, seed weight	1,2
Deshmukh et al. 1998	8	Grain yield, seed weight	1,2, 4
Mhase et al. 1998	23	Grain yield and components	1,2

1. bi: slope of genotype vs site mean regression.
2. S^2di: deviation mean squares for genotypes vs site mean regression.
3. Wi: Wricke's ecovalence.
4. ANOVA: main effects partitioned (ie. environments into years and location, genotypes into desi and kabuli), and used to partition G x E interaction.
5. AMMI: additive main effects and multiplicative interaction.
6. HC: hierarchial clustering.
7. PCA: principal components analysis.

Table 2.7 (continued) Chickpea g x e studies on agronomic traits published since 1971 - organized by publication year and methodology employed to analyse G x E interaction.

Reference	Genotypes	Traits studied	Method employed
Popalghat et al. 1999	19	Grain yield and components	1,2
Khorgade et al. 2000	16	Grain yield and components	1,2
Yadava et al. 2000	81	Grain yield, wilt resistance	1,2
Ereifej et al. 2001	4	Grain yield and seed composition	4
Sood et al. 2001	33	Grain yield, yield components and protein content	1,2, 4
Arshad et al. 2003	25	Grain yield and components	1,2,5
Rubio et al. 2004	11	Grain yield, seed weight, seed size, growth habit, single/double pod, early/late flowering genes	4,5,7
Berger et al. 2004	73	Grain yield, seed weight, growth habit, maturity	5,7
Berger et al. 2006	46	Grain yield, seed weight, growth habit, maturity	1
Berger et al. 2008	619,562	Grain yield, seed weight, growth habit	1
Segherloo et al. 2008	17	Grain yield and components	6
Atta et al. 2009	14	Grain yield and components	4,6
Alwawi et al. 2010	7	Grain yield and components	4
Dehghani et al. 2010	17	Grain yield and components	1,5,7

1. bi: slope of genotype vs site mean regression.
2. S^2di: deviation mean squares for genotypes vs site mean regression
3. Wi: Wricke's ecovalence.
4. ANOVA: main effects partitioned (ie. environments into years and location, genotypes into desi and kabuli), and used to partition G x E interaction.
5. AMMI: additive main effects and multiplicative interaction.
6. HC: hierarchial clustering.
7. PCA: principal components analysis.

$h^2 = \sigma^2_{GE} / \sigma^2_P$

where

$\sigma^2_{GE} = (MS_{GE} - MSe) / r$

$\sigma^2_P = \sigma^2_G + (\sigma^2_{GE}/e) + (\sigma^2 e/re)$

$\sigma^2_G = (MS_G - MS_{GE})/re$

Note:

σ^2_{GE} is the genotype-environment variance

MS_{GE}, mean square of genotype-environment

$\sigma^2 e$ = MSe, the error mean square

σ^2_G, genotypic variance

MS_G, genotypic mean square

e = number of environments

r = replications per environment

For two environments, heritability of plasticity is the ratio of variance components or slope of a parent-offspring regression (Scheiner and Lyman, 1989):

$OMD = h^2 PMD$ + intercept,

Where PMD is the mean difference in the F1 offspring of the parental families and OMD, mean difference in the F2 offspring of the F1 families.

Heritability, like repeatability is specific for given set of genotypes and environments.

2.11 Hypotheses for thesis

The main research thrust with pulse starch concerns its use as a food source with health benefits. Storage components of seeds including starch are affected by the environment (Al-Karaki and Ereifej, 1997). There is little knowledge as to why legume starches digest more slowly than cereal starches, however, knowledge on the relationship between starch chemical and granular structure and starch nutritional characteristics on one hand, and genetic control of starch chemical and granular structure on the other will be critical for starch manipulation and

improvement in legumes (Hedley, 2001). Literature published to-date points to the fact that the production of designer starch is a long term objective requiring a multidisciplinary approach that will involve geneticists and plant breeders to provide genetically characterized lines, biochemists to elucidate dynamics in starch biosynthesis, analytical chemists to determine the changes in molecular structure of starch, physical chemists and physicists to comprehend and interpret starch granular structure and interactions within the granule that contribute to functional properties of starch. The hypotheses for the thesis are therefore:

1. Carbohydrate based seed quality traits have low repeatability, as storage components are strongly influenced by environment.
2. Genetic variability for carbohydrate based seed quality traits is present in natural germplasm collections.
3. There is an association between rate of starch hydrolysis and seed starch structure.

CHAPTER 3

GENOTYPE AND GROWING ENVIRONMENT INFLUENCE CHICKPEA (*CICER ARIETINUM* L.) SEED COMPOSITION

3.1 ABSTRACT

As a first step towards genetic improvement of seed quality in chickpea (*Cicer arietinum* L.), seven desi and nine kabuli varieties were grown at multiple sites in Saskatchewan and Alberta, Canada to assess the affect of environment on seed yield, weight and selected seed constituents. The sites were chosen to represent a range of environments in chickpea production areas of the Canadian prairies. Genotype × environment interaction effects on starch, amylose and protein (desi only) concentrations and seed yield were significant, suggesting that the varieties did not perform consistently relative to each other in the different environments. Starch concentration was negatively correlated ($r_{kabuli} = -0.25$, $P < 0.05$; $r_{desi} = -0.16$, $P < 0.05$) with protein concentration in both chickpea market classes. However, repeatability estimates of starch, amylose and protein concentrations were low and inconsistent across chickpea market classes, possibly owing to complex biosynthetic pathways for these constituents. The results suggest that testing for seed constituent traits over a range of environments will be required to improve seed quality in individual chickpea varieties. The best selection strategies for seed constituent improvement in chickpea will be influenced by genotype and genotype × environment interaction for these traits. The negative relationship between seed constituents and yield indicates that selection for chickpea cultivars with desired seed composition may require compromise and indirect selection.

3.2 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop, ranking second in growing area (15.3% of total pulse area) and third in production (14.6% of total pulse production) around the world (Knights et. al., 2007). It is a staple food in India, North and East Africa, southern Europe and the Americas, with annual production of 8.4×10^6 t from an area of 10.4×10^6 ha (FAO, 2005). Chickpea is an important crop in Saskatchewan, with a total area of production of over 1.28×10^5 ha in 2006 (McVicar et al., 2006; SAF, 2007). Saskatchewan accounts for over 80% of Canadian chickpea production, with the remainder coming from Alberta (SAF, 2007). Two

main chickpea market classes are recognized, kabuli (white flower with large, cream-coloured seeds) and desi (purple flower with smaller, angular, dark-coloured seeds). Desi chickpeas have a thicker testa, accounting for 150 g kg^{-1} dry weight as compared with 70 g kg^{-1} in kabuli chickpeas, which affects the determination of seed composition (Wood and Grusak, 2007; Knights and Mailer, 1989). The mean seed yield of Saskatchewan grown chickpea is 1550 kg ha^{-1} for desi and 1300 kg ha^{-1} for kabuli (McVicar et al., 2006). Chickpea is consumed as whole seed, dhal (decorticated split cotyledons) or dhal flour. In almost three-quarters of the Indian subcontinent, chickpea is utilized as either dhal, dhal flour or whole seeds, whereas in Canada, Australia, Ethiopia, Mexico, Sudan, Tanzania, Turkey, the USA and the UK it is consumed as whole seed (Saini and Knights, 1984). Seed legumes are a good source of complex carbohydrates, proteins and dietary fibre (Wood and Grusak, 2007; Chibbar et al., 2004).

Starch is the major storage carbohydrate and a primary energy source in most human diets and animal feeds. Starch is a complex macromolecule composed of two glucan polymers, an essentially linear chain amylose and a highly branched amylopectin. Starch is normally made up of one quarter amylose and three-quarters amylopectin. The proportions of amylose and amylopectin and their structures vary with the plant species. Chickpea starch concentration varies from 430 to 590 gkg^{-1} seed weight, and total starch concentration is an average of 8% higher in kabuli chickpea than in desi chickpea (Wood and Grusak, 2007; Chibbar et al., 2004). A considerable variation in amylose concentration (310–450 g kg^{-1}) has been reported in chickpea (Wood and Grusak, 2007; Chibbar et al., 2004). In contrast to starch, protein concentration generally varies by only a small magnitude between desi and kabuli market classes (McVicar et al., 2006). Chickpea protein concentration ranges from 160 to 300 g kg^{-1} and from 120 to 290 g kg^{-1} for desi and kabuli market classes respectively and is typically two- to-threefold higher than the protein concentration in cereal seeds (Wood and Grusak, 2007). Chickpea proteins are limited in methionine and cysteine, sulfur containing amino acids, but are high in lysine, making chickpea an ideal companion to cereals that are known to be higher in sulfur amino acids but limited in lysine (Wood and Grusak, 2007).

Seed storage constituents such as protein and starch are affected by both genetic make-up and the environment (Hucl and Chibbar, 1996; Morris, 2004). Several reports have shown the effect of genotype × environment interaction on chickpea yield and some agronomic characters (Berger et al., 2007). An extensive study of 23 chickpea genotypes grown at 37 (1985–1986) and

(1986–1987) locations in western Asia, North Africa, Mediterranean Europe and Latin America revealed significant differences in seed yield (Malhotra, and Singh, 1991). Combined analysis of variance showed the presence of highly significant differences due to location as well as genotype × location and genotype × location × year interactions. Similarly, in another recent study, 46 genotypes (41 Indian, three Australian and two of Mediterranean basin origin) were grown over three years at seven locations in diverse chickpea growing regions of India (Berger et al., 2006). Highly significant genotype × environment interaction for yield was observed, with the interaction accounting for more variance than attributed to genotype alone. Several agronomic traits and yield were evaluated over three years and the genotypes were grouped into five clusters based on their performance. In another study on three chickpea genotypes grown under semi-arid Mediterranean conditions, several seed composition traits were affected by cultivar × growing season interaction (Ereifej et al., 2001). However, no effect was found on seed starch and sodium concentrations. Significant genotype × environment interaction was also reported for chickpea canning quality characteristics (Nleya et al., 2002). In a recent report, chickpea genotype × trial interaction was also found to affect milling parameters such as dehulling efficiency and splitting yield, which are important considerations in chickpea utilization (Wood et al., 2008). Seed storage compounds such as protein and starch are important determinants of chickpea utilisation and processing, including milling. In this study we evaluated seven desi and nine kabuli chickpea varieties at multiple sites to study the effect of environment on seed yield, thousand-seed weight and protein, starch and amylose concentrations.

3.3 MATERIALS AND METHODS

3.3.1 Varieties and environments

Seven desi and nine kabuli varieties of chickpea were grown at nine sites (two in 2004, seven in 2005) in Saskatchewan and two sites (2005) in Alberta (Table 3.1). The experimental sites (environments) represent a range of chickpea production areas of the Canadian prairies (Table 3.1). The Brown (having brownish A horizons and drier) and Dark Brown (having darker A horizons and moister) soil types there are characterized by increased soil organic matter contents. Average precipitation varied from 203 mm in Hodgeville (2005) and Swift Current (2005), two sites in close proximity, to 380 mm in Brooks (2005). Most of the precipitation at all locations was in the month of June, except in Scott (2004) where highest precipitation was recorded in

July (Table 3.1). Mean temperatures varied from 13.4 to 16.5 °C. May recorded lowest temperatures, while mean temperatures in the other three months did not vary significantly (Table 3.1).

The chickpea varieties selected for this study were either adapted cultivars or advanced breeding lines that are likely to become cultivars in the near future. All will be referred to as 'varieties' in this section of the thesis. Detailed information on varieties is given in Table 3.2.

Chickpea is best adapted to a long, warm growing season and is preferably planted in early May and harvested by mid-September in western Canada. Desi and kabuli experiments were separately arranged in a randomised complete block design with three replications per location. Plot size was 4.45 m^2 with four rows per plot, inter-row spacing was 30 cm, row length was 3.65 m and seeding rate was 54 seed m^{-2}. Experiments were carried out under rain-fed conditions, and weed control was done by hand hoeing.

3.3.2 Seed weight

Thousand-seed weight was determined by counting 250 seeds (120 g kg^{-1} moisture content) using an electronic seed counter (Seedburo Equipment Co., Chicago, IL, USA) and a convertible electronic computerized balance.

3.3.3 Dehulling of desi and grinding of chickpeas

Desi chickpeas were dehulled for 60 s using a Satake seed testing mill (Satake Engineering Company, Tokyo, Japan) equipped with a Satake abrasive roller stone driven by an electric motor. A Turkish table-top dehuller (BuffaloMachines, Buffalo, NY, USA) was used to separate the hulls from the cotyledons. Intact kabuli and dehulled desi chickpea seeds were broken into small pieces and ground into a fine meal using a UDY cyclone mill (UDY Corporation, Fort Collins, CO, USA) to pass through a 0.5 mm sieve.

3.3.4 Total starch

Total starch concentration was determined according to the Megazyme method (Megazyme International Ireland Ltd, Wicklow, Ireland) (McLeary et al. 1994). Briefly, a 100 mg sample was suspended in 0.2 mL of 800 mL L^{-1} ethanol to aid dispersion and mixed vigorously using a vortex mixer. The sample was incubated at 100 °C for 8 min and mixed every 2 min with 300 U of α-amylase (AA) in 50 mmol L^{-1} 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (pH

Table 3.1. Soil zone, monthly precipitation (P, mm) and mean temperature (T,◦C) during growing period at trial locations (data obtained from http://www.climate.weatheroffice.ec.gc.ca/).

Environment				Growing period (month)				
Location (province[a])	Year	Soil zone		May	June	July	Aug	TP/MT[b]
Bow Island (AB)	2005	Brown	P	8	148	3	50	209
			T	8.6	19.2	20.5	18.2	16.4
Brooks (AB)	2005[c]	Brown	P	23	228	42	87	380
			T	8.3	17.4	18.5	17.8	15.4
Hodgeville (SK)	2005[d]	Brown	P	29	97	27	50	203
			T	9.1	18.6	17.6	20.0	16.2
Swift Current (SK)	2005	Brown	P	29	97	27	50	203
			T	9.1	18.6	17.6	20.0	16.2
Davidson (SK)	2005	Dark Brown	P	73	157	44	40	314
			T	9.9	18.6	17.9	19.3	16.5
Elrose (SK)	2005	Dark Brown	P	44	134	38	34	249
			T	7.1	16.1	14.7	15.6	13.4
Goodale (SK)	2005	Dark Brown	P	31	110	55	61	256
			T	7.8	18.0	17.6	16.5	14.8
Kyle (SK)	2004[c]	Dark Brown	P	51	88	60	72	271
			T	9.5	15.2	16.3	14.9	14.2
Kyle (SK)	2005	Dark Brown	P	57	165	21	31	275
			T	8.8	18.2	20.3	19.2	16.4
Scott (SK)	2004[c]	Dark Brown	P	15	68	99	52	234
			T	8.1	15.6	17.4	13.1	13.6
Scott (SK)	2005[c]	Dark Brown	P	51	88	60	72	271
			T	8.7	16.9	18.1	16.7	15.2

[a]AB, Alberta; SK, Saskatchewan.
[b]TP, total precipitation; MT, mean temperature.
[c]Used for desi only.
[d]Used for kabuli only.

Table 3.2 Description of chickpea varieties used in the study.

Variety	Origin	Pedigree	Maturity[a]
Desi varieties			
Myles	USDA/ARS, USA	BDN 9-3/K1184//ICP 87440	Medium
316B-42	CDC, Canada	Myles//B-90/92040-52B	Medium
CDC Cabri	CDC, Canada	ICCX-860027/ICCX-860047	Medium
CDC Vanguard	CDC, Canada	92073-60D//92056-8/ICCV96029	Medium
ICC-12512-9	Landrace, India	Unknown	Medium
ICC-12512-1	Landrace, India	Unknown	Medium late
CDC Anna	CDC, Canada	ICCX-860047/ICC7002	Late
Kabuli varieties			
FLIP97-133C	ICARDA, Syria	Unknown	Medium late
FLIP98-135C	ICARDA, Syria	Unknown	Medium late
FLIP98-134C	ICARDA, Syria	Unknown	Medium late
Amit	Landrace, Bulgaria	Unknown	Late
CDC Xena	CDC, Canada	C188-178/ICCV89511	Late
CDC Frontier	CDC, Canada	FLIP91-22C/ICC14912	Late
97-Indian2-1	India	Unknown	Late
FLIP97-45C	ICARDA, Syria	Unknown	Late
Sanford	USDA/ARS, USA	FLIP85-58/Surutato-77	Very late

[a] Early, ≤120 days; late, >150 days.

7.0) to hydrolyse starch to dextrins. Subsequent incubation with 4 mL of 20 mmol L^{-1} sodium acetate buffer (pH 4.5) and 0.1 mL of 20 U amyloglucosidase (AMG) at 50 °C for 30 min hydrolysed dextrins to glucose. The reaction mixture was diluted to 100 mL and a 1 mL aliquot was added to 3 mL of glucose determination reagent (GOPOD) and incubated at 50 °C for 20 min. Total starch was calculated as free glucose by measuring the absorbance at 510 nm as described previously (Hucl and Chibbar, 1996). Commercial corn starch (Arancia Corn Products SA de CV, Mexico City, Mexico) provided with the Megazyme kit was used as a standard to accurately determine starch concentration.

3.3.5 Isolation of starch granules

Starch was isolated from chickpea seeds using a previously described procedure (Zao and Sharp, 1996; Demeke et al. 1997). The seeds were cut into several small pieces, placed in 1 mL of sterile distilled water in a 1.5 mL microfuge tube and kept overnight at 4 °C. The water was carefully decanted and a small pestle was used to grind the seeds into slurry. The slurry was layered over 1 mL of 800 g L^{-1} caesium chloride in a 2 mL microfuge tube. The microfuge tube was centrifuged at 13 000 × g for 5 min. The supernatant was discarded and the starch granules pelleted at the bottom were centrifuged once again through caesium chloride to remove any adhering nonstarch molecules. The pelleted starch granules were washed twice with buffer [55 mmolL^{-1} Tris-HCl, pH 6.8, 23 g L^{-1} sodium dodecyl sulfate (SDS), 100 mL L^{-1} glycerol], once with water and a final time with acetone. After the final wash, the starch granules were dried at room temperature and used for amylose concentration determination.

3.3.6 Amylose determination

A high-performance size exclusion chromatography (HP-SEC) method was used to determine amylose concentration (Demeke et al. 1999). A 5 mg starch sample was suspended in 5 mL of distilled water in a glass tube and incubated at 130 °C for 30 min. To 1 mL of this gelatinized starch solution, 55 μL of 1mol L^{-1} sodium acetate (pH 4) was added with vigorous mixing, followed by 4 U of isoamylase to debranch the starch. After 4 h of incubation at 40 °C the debranching reaction was stopped by boiling for 20 min to inactivate isoamylase. The debranched starch solution was freeze dried. The freeze-dried sample was dissolved in 200 μL of 990 mL L^{-1} dimethyl sulfoxide (DMSO) and centrifuged in a microfuge at 15 000 × g. A 40μL

aliquot of the supernatant was injected into a PLgel MiniMix-C guard column attached to a PLgel MiniMix 4.6 mmi.d. column (Polymer Laboratories, Inc., Amherst, MA, USA) to separate amylose and amylopectin using a high-performance liquid chromatography (HPLC) system comprising a Waters 600 controller, Waters 610 fluid unit, Waters 717 Plus autosampler and Waters 410 differential refractometer (Waters Corporation, Milford, MA, USA). The data were collected and analysed using Empower software (Waters Corporation). Starch samples, column and detector were maintained at 40, 100 and 45 °C respectively. The eluent used was 990 mL L^{-1} DMSO at a flow rate of 0.2 mL min^{-1}. The amylose concentration was calculated by integration of the peak area corresponding to amylose with respect to the peak area corresponding to both amylose and amylopectin (Demeke et al. 1999).

3.3.7. Protein concentration

The protein concentration of chickpea seed meal was determined using the combustion method. The ground chickpea sample was passed through hot copper to remove oxygen and to convert NOx into N_2, followed by Lecosorb and Anhydrone treatment to remove carbon dioxide and water; the nitrogen in the sample was measured by the thermal conductivity cells (FP-528 Protein/Nitrogen Analyser, Leco Corporation, St Joseph, MI, USA). The crude protein concentration of each sample was calculated using the following formula (AACC, 2000):

crude protein (%) = % N × 6.25

3.3.8 Statistical analyses

Analysis of variance (ANOVA) was done for each trait and market class in each location followed by a combined analysis across locations. Mean comparison for each trait across varieties was done using Duncan’s multiple range test (DMRT). Phenotypic correlations between traits and P values were estimated using SAS Version 8.0 (SAS Institute Inc., Cary, NC, USA). Location and replication effects were considered random and the genotypic effect as fixed in a mixed model (PROC MIXED) of the SAS program. Principal component analysis (PCA) was done using SYSTAT software Inc., Chicago, IL, USA for Windows Version 12 on the mean value of each trait across environments. All differences were significant at $P \leq 0.05$ unless noted otherwise.

3.3.9 Repeatability estimates

Repeatability (r_{approx}) was calculated from the ANOVA based on the F ratio and its degree of freedom (Lessells and Boag, 1987):

$n = (df1 + df2 + 1)/(df1 + 1)$

$rapprox = (F - 1)/(F - 1 + n)$

where n is the mean group size, df1 is the numerator degree of

freedom, df2 is the denominator degree of freedom and F is the F

ratio value of the trait.

3.3.10 Heritability estimates

Heritability of clonal replicates or full-sibs grown in multi-environments was estimated from mean square values of ANOVA table as the ratio of genotype-environment interaction variance to the total phenotypic variance (Scheiner and Lyman, 1989; Becker, 1984). Thus

$h^2 = \sigma^2_{GE} / \sigma^2_P$

where $\sigma^2_{GE} = (MS_{GE} - MSe)/r$

$\sigma^2_P = \sigma^2_G + (\sigma^2_{GE}/e) + (\sigma^2 e/re)$

$\sigma^2_G = (MS_G - MS_{GE})/re$

$\sigma^2 e = MS_E$, the error mean square

e = number of environments

r = replications per environment

3.4 RESULTS AND DISCUSSION

Significant genotype, environment and genotype × environment interaction effects were detected for seed yield, seed weight, starch, amylose and protein concentrations in both desi and kabuli varieties (Table 3.3.). In kabuli varieties, protein × location interaction was not significant (Table 3.3). The results suggest adequate variability for studied characters among genotypes and

genotypic plasticity over environments. Chickpea market class (desi versus kabuli) had a consistent effect on total starch concentration (see Tables 3.4 and 3.5). Genotype × environment interactions for thousand-seed weight and yield have been reported (Malhotra and Singh, 1991; Sood et al. 2001; Berger et al. 2006). A cluster analysis of genotype × environment interaction was used as a tool to classify chickpea growing environments and develop guidelines for inclusion of varieties in international nurseries for testing (Malhotra and Singh, 1991). Analysis of genotype × environment interaction in 46 genotypes grown at seven sites over three years revealed both specific and wide adaptation in chickpea to low- and high-yielding environments. Starch and amylose concentrations showed genotype × environment interaction in both desi and kabuli varieties, but protein concentration did so only in desi varieties (Table 3.3). Seed composition traits are less affected by genotype × environment interaction as compared with thousand-seed weight and yield (Ereifej et al. 2001; Singh et al. 1983; Singh et al. 1993).

3.4.1 Desi chickpea

For desi varieties, mean seed yield across varieties and environments was 1462 kg ha^{-1} and mean thousand-seed weight was 228 g (Table 3.4). ICC-12 512-9 and Myles had the highest (1710 kg ha^{-1}) and lowest (1120 kg ha^{-1}) yields respectively. Myles also had the lowest thousand-seed weight, while CDC Cabri had the highest thousand-seed weight but was a medium-yielding variety (Table 3.4). Significant genotype × environment interactions were observed for seed yield and thousand-seed weight (Table 3.3). CDC Vanguard and CDC Anna had the lowest protein concentrations, while Myles, which had the lowest-thousand seed weight and was lowest-yielding, had the highest protein concentration (Table 3.4). Desi chickpeas have a thick seed coat (Wood and Grusak, 2007; Knights and Mailer, 1989) which might skew the seed composition results. Moreover, desi chickpeas are consumed as dhal produced after dehulling. Therefore dehulled desi chickpeas were used for seed composition analysis. Total starch concentration showed less variation, with desi varieties having total starch concentrations of 420–452 g kg^{-1}, with a mean of 435 g kg^{-1} (Table 3.4), similar to values reported previously (Bakhsh et al. 2006; Wood et al. 2008). CDC Cabri, CDC Anna, CDC Vanguard and ICC-12 512-9 had the highest total starch concentrations (Table 3.4). Interestingly, CDC Cabri had the highest starch concentration across all environments. Amylose concentration showed less variation among desi

Table 3.3 Mean squares of combined ANOVA and coefficient of variation (CV) for seed yield, seed weight and protein, starch and amylose concentrations of desi and kabuli chickpea varieties grown in different environments in western Canada.

	Variety (G)	Location (E)	G x E	CV (%)
Desi				
Seed yield	1250000**	7890000**	200000**	12.2
Seed weight	42800**	2900**	400**	5.5
Protein	2400**	17500**	159**	4.1
Starch	3200**	41600**	1500**	7.0
Amylose	563*	1540**	186**	2.3
Kabuli				
Seed yield	1309000**	4860000**	88260**	12.7
Seed weight	59640**	22200**	2060**	10.0
Protein	1150*	8117**	478ns	12.8
Starch	6894**	22185**	3415**	4.3
Amylose	175**	836**	385**	2.4

*Significant at 5% level; **Significant at 1% level; ns, not significant.

Table 3.4 Seed yield, seed weight and protein, starch and amylose concentrations of desi chickpea varieties evaluated across different environments in western Canada.

	Seed yield (kg ha^{-1}	Seed weight (g)	Protein (gkg^{-1} seed meal)	Starch (gkg^{-1} seed meal)	Amylose(gkg^{-1} starch)
Variety					
ICC-12512-9	1710a	260.8b	192.2cd	439.1ab	267.8cd
ICC-12512-1	1690a	245.4c	191.6de	425.9bc	265.7e
CDC Vanguard	1580b	222.1d	185.4f	438.5ab	269.9bc
CDC Cabri	1410c	286.9a	196.3c	451.6a	263.8e
316B-42	1390c	199.3e	200.7b	431.0bc	266.4de
CDC Anna	1270d	197.0e	187.7ef	439.6ab	276.4a
Myles	1120e	184.5f	211.5a	420.2c	272.3b
Mean	1462	228.0	195.1	435.1	268.9
CV	12.2	5.5	4.1	7.0	2.2
Pooled SE	32.7	2.3	0.2	0.6	0.1
Location					
Brooks 2005	2690a	233.1bc	217.8b	410.9d	251.2f
Bow Island 2005	2210b	232.0bc	184.0e	450.4b	263.6e
Goodale 2005	1614c	208.0f	176.9f	449.0b	268.4d
Davidson 2005	1550c	216.0e	154.7g	429.0cd	274.2bc
Kyle 2004	1430d	218.0ed	190.0d	477.3a	264.4e
Kyle 2005	1380d	250.0a	197.4c	475.3a	279.3a
Swift Current 2005	1204e	235.0b	212.7b	417.6d	271.8cd
Elrose 2005	1034f	230.0bc	155.5g	438.5bc	277.9ab
Scott 2004	880g	231.0bc	245.6a	475.3a	275.0bc
Scott 2005	630h	225.7cd	216.5b	328.1e	263.1e
Mean	1462	228.0	195.1	435.1	268.9
CV	8.5	3.7	3.2	5.1	2.2
Pooled SE	39.1	2.7	0.2	0.7	0.1

Means followed by the same letter within a column are not significantly different at $P<0.05$ based on DMRT.

varieties. The mean amylose concentration of desi varieties was 269 g kg^{-1} starch (Table 3.4). CDC Anna had the highest amylose concentration, followed by Myles and CDC Vanguard. In general, amylose concentration in both desi and kabuli chickpeas is lower than that reported in other studies (Saini and Knights, 1984; Chibbar et al. 2004; Wood and Grusak, 2007). In this study, debranched starch was separated using an SEC-HPLC method, which determined the amount of amylose molecules (Demeke et al. 1999) compared with iodine-based spectrophotometric methods determining apparent amylose concentration.

In desi chickpea varieties, growing environment significantly affected all traits studied. Interestingly, high precipitation during vigorous plant growth (June) resulted in higher yields. Brooks with 228 mm precipitation in June recorded the highest yield (2690 kg ha^{-1}), while Scott 2004 and 2005 with only 68 and 88 mm precipitation in June, respectively, recorded the lowest yields (630 and 880 kg ha^{-1} respectively) (Table 3.4). The amount of June precipitation effected extreme values of seed yield in desi chickpea varieties, but seed yield is the net result of several interacting factors, as shown by observations with intermediate June precipitation levels (Table 3.4). Thousand-seed weight was highest at Kyle 2005 (250 g) and lowest at Goodale (208 g) (Table 3.4). Kyle 2004, Kyle 2005 and Scott 2004 had the highest seed starch concentrations. Scott 2005 had the lowest total starch concentration (Table3. 4). Kyle 2005 had the highest amylose concentration of 279 g kg^{-1} starch. This may be attributed to the inherent properties of local edaphic factors as well as the lower temperatures (Table 3.1) compared with other locations. Brooks recorded significantly ($P < 0.05$) lower amylose concentration (251 g kg^{-1} starch) (Table 3.4).

3.4.2 Kabuli chickpea

Kabuli varieties had lower seed yield (Table 3.5) compared with desi varieties (Table 3.4). CDC Frontier had the highest seed yield, which was not significantly different from those of FLIP97-133C, Amit and FLIP97-45C. Sanford produced the lowest yield of 860 kg ha^{-1}. CDC Xena and FLIP98-135C showed the highest thousand seed weights, 449 and 427 g respectively. Kabuli varieties had an average of 186 g kg^{-1} protein, which is slightly lower as compared with desi varieties. Protein concentration did not differ greatly among kabuli varieties (Table 3.5).

In general, kabuli chickpea varieties had higher total starch concentration than desi varieties (Tables3.4 and 3.5), which may be due to the larger seed size of kabuli (8–10 mm)

compared with desi (<8 mm). Moreover, kabuli chickpeas were used intact with the hulls (testa), contributing 6.7% of dry weight and 5.6% of dietary fibre in kabuli chickpea, which is half the contribution to seed dry weight in desi chickpea (Knights and Mailer, 1989). Desi chickpeas were dehulled prior to milling, because their hulls are easy to remove as compared with those of kabuli chickpeas. Kabuli varieties had total starch concentrations of 480–550 g kg^{-1} meal, with a mean of 546 g kg^{-1} meal (Table 3.5). These starch concentrations are similar to those reported previously (Saini and Knights, 1984; Jambunathan and Singh, 1980). FLIP97-133C produced the highest, while Amit yielded significantly ($P < 0.05$) lower starch concentration. The other varieties had similar starch concentrations (Table 3.5). FLIP97-133C was the highest starch yielder at Elrose, Davidson and Goodale but the lowest starch yielder at Kyle.

Kabuli chickpea varieties had higher amylose concentration than desi varieties (Tables 3.4 and 3.5). The amylose concentration in this study is comparable to the 26.8–29.0% reported by Singh et al. (1956) but lower than and not consistent with the 33.5 and 36.3% for kabuli and desi varieties respectively reported by Saini and Knights (1984). The amylose concentration of kabuli chickpea varieties ranged from 271 to 280 g kg^{-1} starch (Table 3.5).

For kabuli varieties, Bow Island was the best location for seed yield, with an average of 2224 kg ha^{-1}, while the lowest seed yield of 910 kg ha^{-1} was obtained at Hodgeville (Table 3.5). Interestingly, similar to desi chickpea varieties, the highest yield was recorded at Bow Island, which had the highest amount of June precipitation (148 mm, Table 3.1). However, no definite trends were observed with other locations. Kyle produced the highest thousand-seed weight of 417 g, whereas Davidson and Goodale produced the lowest thousand-seed weights. The effect of genotype × environment on both seed yield and thousand seed weight was significant ($P < 0.05$) (Table 3.3), indicating that these respective traits ranked differently from one environment to another.

Average protein, starch and amylose concentrations of kabuli varieties across environments were 186, 547 and 276 g kg^{-1} meal respectively. For kabuli varieties, Swift Current had the highest protein concentration at 210 g kg^{-1}, whereas Davidson yielded the lowest protein concentration at 159 g kg^{-1}. Elrose recorded the highest total starch concentration at 565 gkg^{-1} meal and Swift Current the lowest at 484 g kg^{-1} meal (Table 3.5). Amylose concentration ranged from 265 g kg^{-1} starch at Elrose to 280 g kg^{-1} starch at Davidson, Goodale and Hodgeville (Table 3.5).

3.4.3 Trait correlation

A positive correlation ($r = 0.16$, $P = 0.05$) was detected between starch concentration and seed yield in desi varieties, but there was no association ($r = -0.01$, ns) in kabuli varieties. The relationship between starch concentration and seed weight was positive in both kabuli ($r = 0.16$, $P = 0.05$) and desi ($r = 0.11$, ns) varieties but was not significant in the latter. Starch and protein concentrations were negatively correlated in desi ($r = -0.16$, $P = 0.05$) and kabuli ($r = -0.25$, $P = 0.01$) varieties. In desi varieties, amylose concentration was positively correlated with starch concentration ($r = 0.18$, $P = 0.01$) but negatively correlated with seed weight ($r = -0.16$, $P = 0.02$), protein concentration ($r = -0.16$, $P = 0.02$) and seed yield ($r = -0.40$, $P = 0.01$) (Table 3.6). The generally small magnitude of the correlation between traits may have been due to the relatively small sample size used and/or the relatively narrow genetic base of the genotypes.

In kabuli varieties, amylose concentration had a significant ($r = -0.21$, $P = 0.01$) negative relationship with starch concentration but not with protein concentration ($r = 0.07$, ns), seed yield ($r = -0.05$, ns) and seed weight ($r = 0.02$, ns) (Table 3.6). This implies that selecting for high-amylose desi will require selecting for high starch yield, whereas selecting for high amylose kabuli will mean selecting for low starch yield (Hildebrand, 1990). The relationship between amylose and starch concentrations in desi is similar to that reported in potatoes (XinLing et al., 2005). Protein concentration and seed yield were negatively correlated ($r = -0.19$, $P = 0.01$) in desi but not in kabuli ($r = -0.06$, ns). No correlation was observed between protein concentration and seed weight in desi ($r = 0.04$, ns) and kabuli ($r = -0.02$, ns) chickpeas.

Genotype × environment interaction trial data can be partitioned as genotype × environment (location) data for traits being studied (Figures 3.1– 3.12) and as genotype × trait data on individual environments (Figures. 3.13-3.16). PCA plots provide an effective tool for visual analysis of two way data. Both varieties and environments will be scattered in all directions in PCA plots if there is a minor effect of genotype in the data, and PC1 and PC2 will be dominated by genotype × environment. This statement may be supported by low repeatability values (see Table 3.7). On the other hand if genotype is sizable, PC1 will be dominated by genotype and all other PCs will be dominated by genotype × environment. Figures 3.1 and 3.4 both show two non-overlapping clusters of varieties and three non-overlapping clusters of environments for amylose concentration in desi chickpea. This clustering of environments reflects genotype × environment (location) interactions for amylose concentration (Table 3.3). Genotype ×

environment interaction trial data can be partitioned as genotype × environment (location) data for traits being studied (Figures 3.1– 3.12) and as genotype × trait data on individual environments (Figures 3.13-3.16). PCA plots provide an effective tool for visual analysis of two way data. Both varieties and environments will be scattered in all directions in PCA plots if there is a minor effect of genotype in the data, and PC1 and PC2 will be dominated by genotype × environment. This statement may be supported by low repeatability values (see Table 3.7). On the other hand if genotype is sizable, PC1 will be dominated by genotype and all other PCs will be dominated by genotype × environment. Figures 3.1 and 3.4 both show two non-overlapping clusters of varieties and three non-overlapping clusters of environments for amylose concentration in desi chickpea. This clustering of environments reflects genotype × environment (location) interactions for amylose concentration (Table 3.3). According to Yan and Tinker (2005), the total variation explained by a PCA plot determines its credibility, and genotype × environment for the trait may be complex if the plot explains only a small fraction of the total variation. For instance, Figure 3.1 explained that 77% of the total variation in amylose concentration was due to genotypic effects as compared with 66% (Figure 3.4) due to environmental effects.

Positive genotype × environment can be exploited and negative genotype × environment avoided by dividing environments into meaningful clusters and deploying different cultivars for different environments. The average environmental coordination (AEC) (Yan, 2001) abscissa has one direction with an arrow pointing to greater genotype main effect. The AEC ordinate is indicated by a double arrow, which when pointed in either direction away from the biplot origin indicates greater genotype × environment effect and reduced stability. CDC Anna was the most stable and high yielding cultivar for both amylose and starch concentrations in most environments (Figures 3.1 and 3.2), while 316B-42 was the most stable and high-protein cultivar (Fig. 3.3). Elrose produced one of the highest amylose concentrations and was the most stable environment for that trait (Figure 3.4). Davidson and Brooks were stable environments that produced high desi starch concentration (Fig. 3.5), but this is inconsistent as their mean values are below average (Table 3.4). According to Yan (2002), this inconsistency is because biplots do not explain 100% of genotype and genotype × environment interaction. Scott 2005 and Brooks were the most stable and high-yielding environments for protein concentration (Figure 3.6). The relative dispersion of the varieties suggests a high level of genetic diversity for amylose concentration compared with

Table 3.5 Seed yield, seed weight and protein, starch and amylose concentrations of kabuli chickpea varieties evaluated across environments in western Canada.

	Seed yield (kg ha^{-1}	Seed weight (g)	Protein (gkg^{-1} seed meal)	Starch (gkg^{-1} seed meal)	Amylose(gkg^{-1} starch)
Variety					
CDC Frontier	1620a	361.2f	187.9a	520.4b	274.0bcd
FLIP97-133C	1574ab	366.4ef	171.2b	549.5a	277.5ab
Amit	1555abc	255.1g	191.8a	480.0c	271.1d
FLIP97-45C	1517abcd	376.7ed	189.7a	525.8b	272.8cd
FLIP98-135C	1490bcd	427.4b	186.2ab	526.1b	277.0abc
FLIP98-134C	11450cd	384.4cd	183.7ab	526.8b	275.9abc
97-Indian2-1	1430d	392.0c	187.3ab	516.1b	280.4a
CDC Xena	1100e	449.0a	182.0ab	524.1b	275.0bcd
Sanford	860f	380.0cd	198.1a	516.9b	278.1ab
Mean	1398	376.6	186.4	546.6	275.7
CV	12.7	5.4	12.8	4.3	2.4
Pooled SE	38.7	4.4	0.5	0.5	0.2
Location					
BowIsland 2005	2224a	380.2cd	192.3bc	503.2d	276.4ab
Elrose 2005	1435b	390.4cb	172.0d	565.3a	264.5c
Kyle 2005	1423b	417.1a	194.2b	499.7d	273.3b
Davidson 2005	1407b	354.6e	158.4e	518.0c	279.8a
Goodale 2005	1390b	327.7f	198.2ab	549.1b	280.1a
SwiftCurrent 2005	980c	375.2d	209.7a	483.9e	276.1ab
Hodgeville 2005	910c	393.2b	180.3cd	525.3c	280.1a
Mean	1398	376.9	186.4	546.6	275.7
CV	6.4	2.7	7.1	2.0	2.4
Pooled SE	34.1	3.9	0.5	0.4	0.1

Means followed by the same letter within a column are not significantly different at $P<0.05$ based on DMRT.

Table 3.6 Correlation coefficients among chickpea seed yield, seed weight and protein, starch and amylose concentrations evaluated across environments in western Canada.

	Seed yield	Seed weight	Protein	Starch	Amylose
Desi					
Seed yield	1	0.04ns	-0.19**	0.16*	-0.40**
Seed weight			0.04ns	0.11ns	-0.16*
Protein				-0.16*	-0.16*
Starch					0.18*
Amylose					1
Kabuli					
Seed yield	1	0.04ns	-0.06ns	-0.01ns	-0.05ns
Seed weight			-0.02ns	0.16*	0.02ns
Protein				-0.25**	0.07ns
Starch					-0.021**
Amylose					1

*Significant at 5% level.

**Significant at 1% level.

ns, not significant.

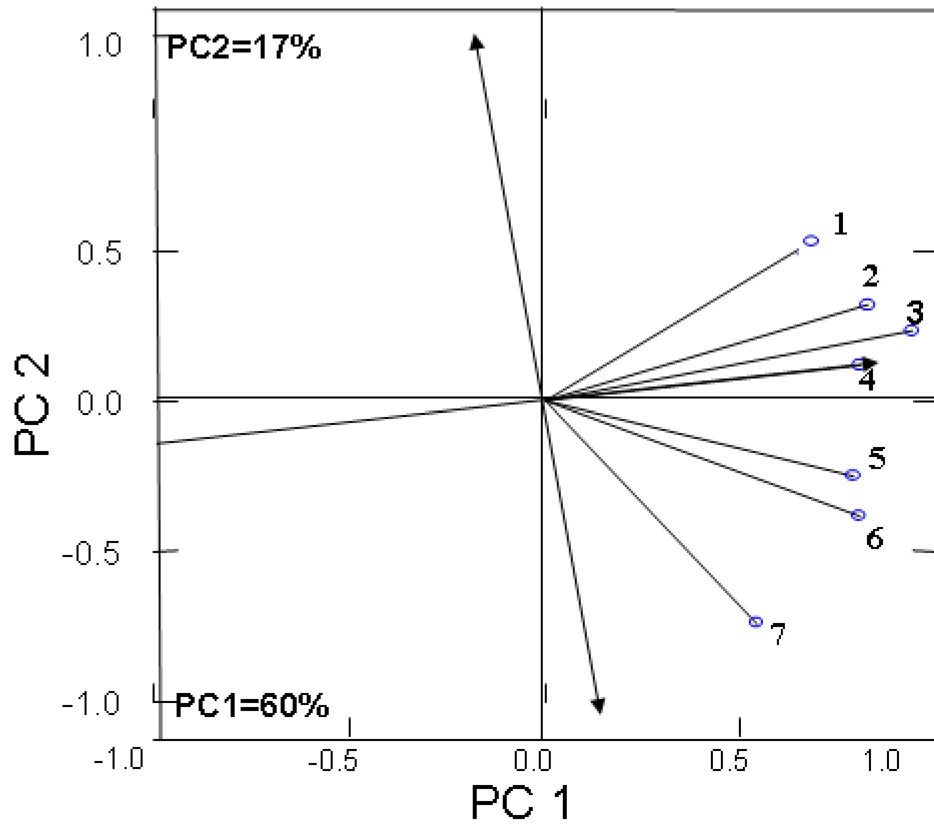

Figure 3.1. Plots of first two major principal components for genotypic effect on major seed components in desi chickpeas, representing genotype-focused, singular value partitioning for amylose: 1, ICC-12512-1; 2, CDC Cabri; 3, Myles; 4, CDC Anna; 5, Vanguard; 6, ICC-12512-9; 7, 316B-42.

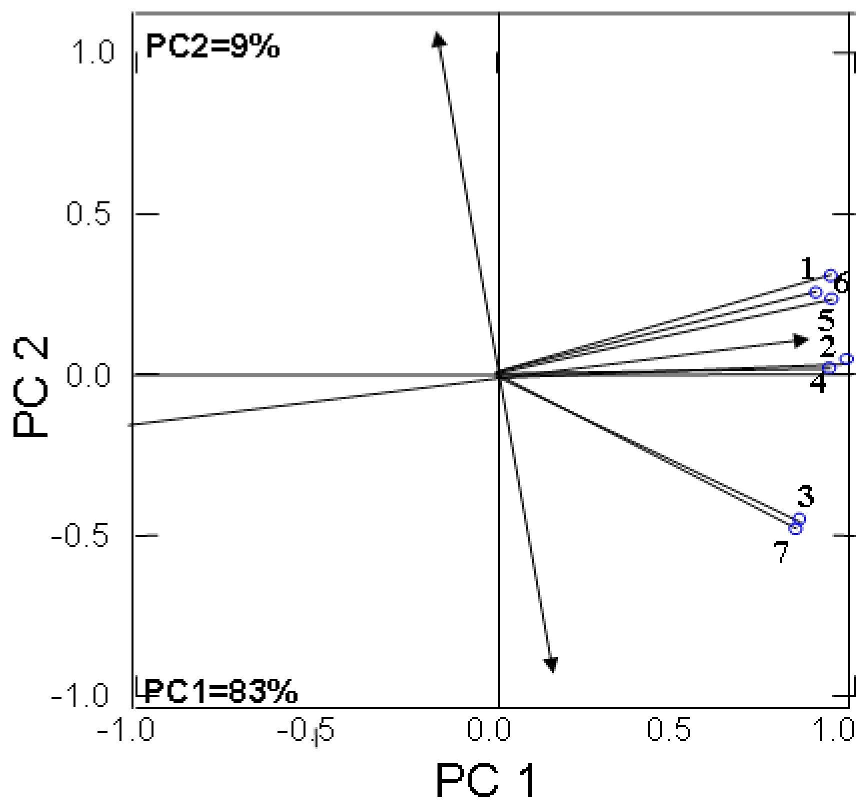

Figure 3. 2. Plots of first two major principal components for genotypic effect on major seed components in desi chickpeas, representing genotype-focused, singular value partitioning for starch: 1, ICC-12512-1; 2, CDC Cabri; 3, Myles; 4, CDC Anna; 5, Vanguard; 6, ICC-12512-9; 7, 316B-42.

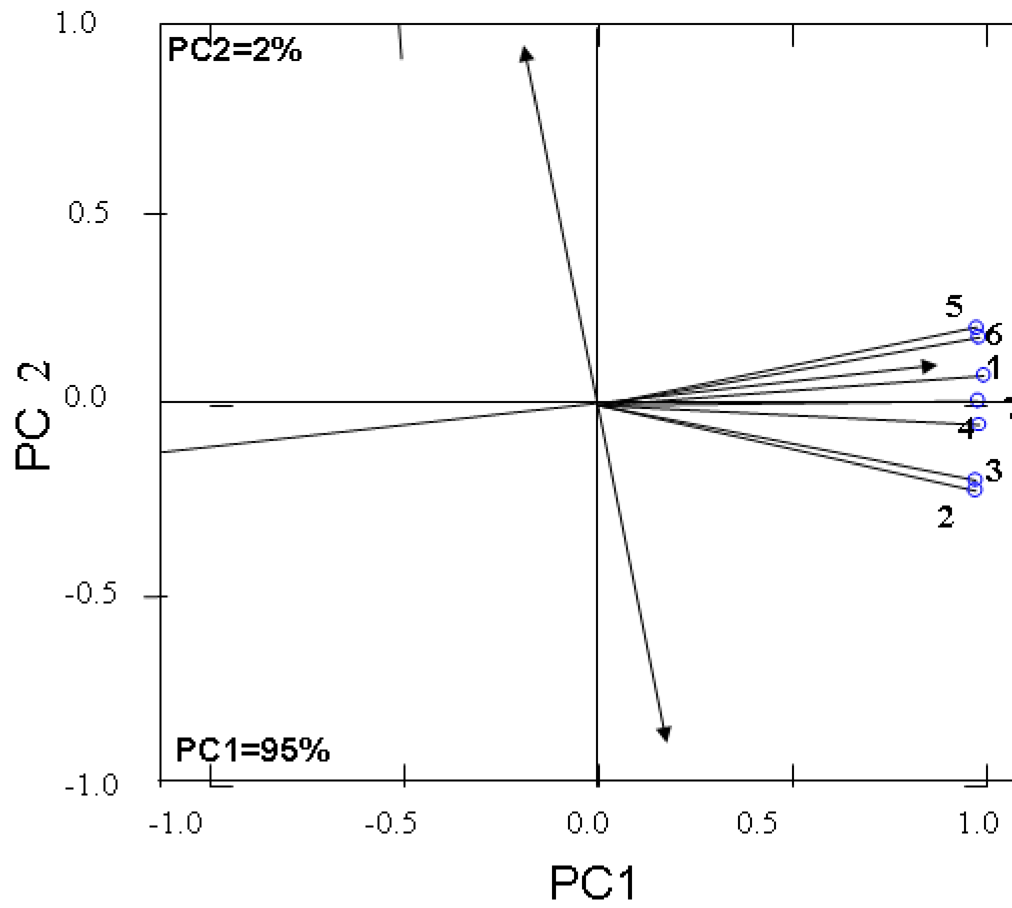

Figure 3.3. Plots of first two major principal components for genotypic effect on major seed components in desi chickpeas, representing genotype-focused, singular value partitioning for protein: 1, ICC-12512-1; 2, CDC Cabri; 3, Myles; 4, CDC Anna; 5, Vanguard; 6, ICC-12512-9; 7, 316B-42.

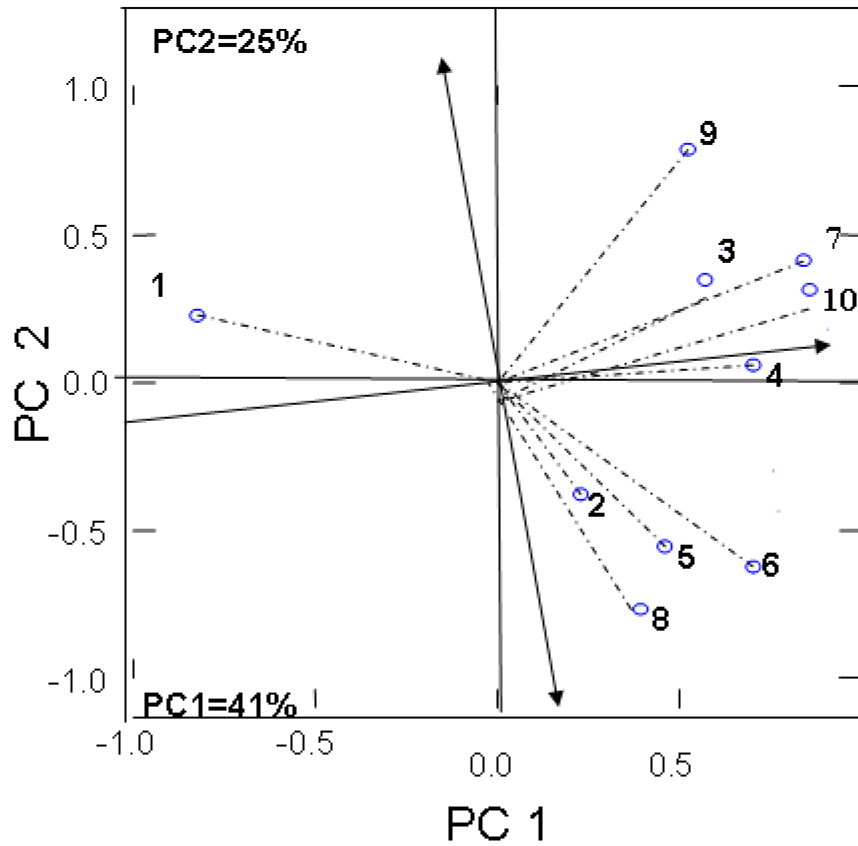

Figure 3.4. Plots of first two major principal components for environmental effect on major seed components in desi chickpeas, representing environment focused, singular value partitioning for amylose: 1, Bow Island; 2, Brooks; 3, Davidson; 4, Elrose; 5, Goodale; 6, Kyle 2004; 7, Kyle 2005; 8, Scott 2004; 9, Scott 2005; 10, Swift Current.

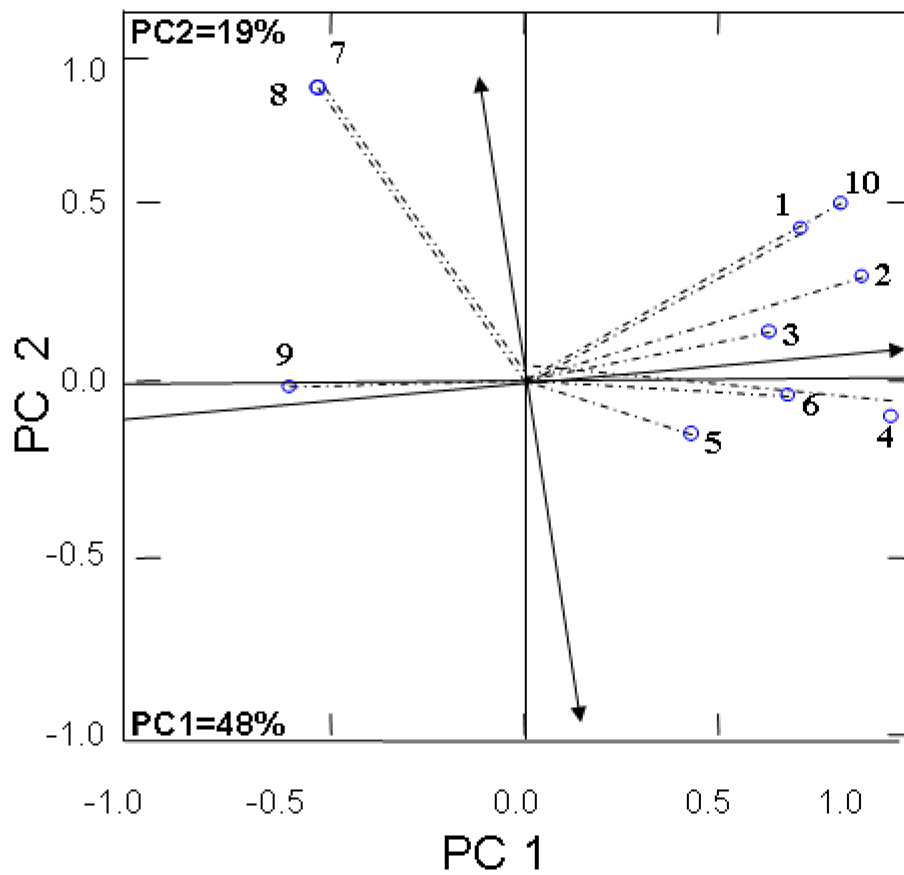

Figure 3.5. Plots of first two major principal components for environmental effect on major seed components in desi chickpeas, representing environment focused, singular value partitioning for starch: 1, Bow Island; 2, Brooks; 3, Davidson; 4, Elrose; 5, Goodale; 6, Kyle 2004; 7, Kyle 2005; 8, Scott 2004; 9, Scott 2005; 10, Swift Current.

.

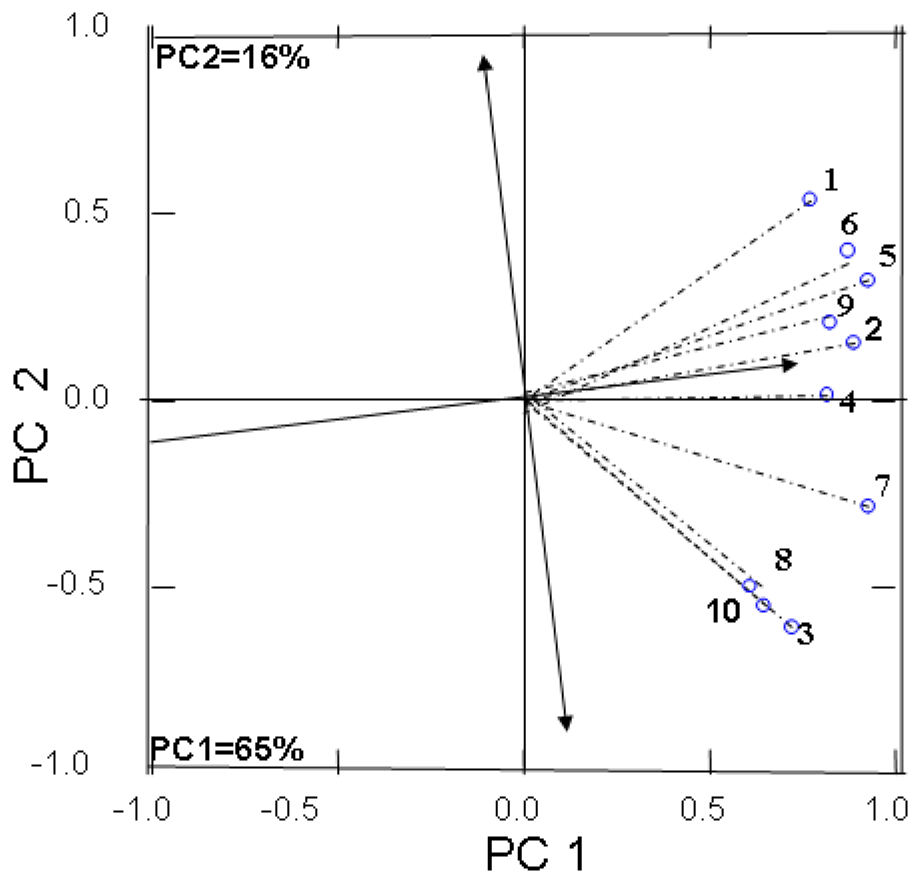

Figure 3.6. Plots of first two major principal components for environmental effect on major seed components in desi chickpeas, representing environment focused, singular value partitioning for protein: 1, Bow Island; 2, Brooks; 3, Davidson; 4, Elrose; 5, Goodale; 6, Kyle 2004; 7, Kyle 2005; 8, Scott 2004; 9, Scott 2005; 10, Swift Current.

starch and protein concentrations in kabuli varieties (Figures 3.7-3.9). FLIP98-135C was among the top performers and the most stable cultivar for amylose and starch concentrations. Sanford was among the most stable for protein concentration. Three non-overlapping clusters of environments were observed in kabuli varieties for starch and amylose concentrations but not for protein concentration (Figures 3.10-3.12).

The clustering of Goodale in the fourth quadrant is inconsistent with results in Table 3.5, because Goodale performed above average for both kabuli amylose and starch concentrations and therefore should have been in the first or second quadrant. Kyle and Hodgeville were among the good but unstable environments for kabuli amylose concentration, contrasting with Goodale and Bow Island. Davidson was the best and most stable environment for amylose concentration, whereas Kyle and Elrose were shown to be the most stable environments for kabuli starch and protein concentrations. The results show that Elrose and Davidson were the best environments and Bow Island the worst environment with respect to desi chickpea amylose concentration (Figure 3.4). In kabuli varieties, Bow Island and Elrose were the best environments, and Goodale and Swift Current the most unstable environments for protein concentration (Figure 3.9).

Finding protein, starch and amylose in different clusters with obtuse angles between their vectors indicates that their effects on quality were independent (Figures 3.13-3.16). Lack of phenotypic correlation among traits (Table 3.6) may be the underlying reason for this. However, the vector lengths for protein, starch and amylose concentrations show that they individually have a pronounced effect on seed quality. Vector length indicates the degree of effect of that trait on seed quality. Short vectors have a minor effect whereas long vectors have a pronounced effect. The traits for both desi and kabuli varieties had both positive and negative values for both axes, implying that trait–quality relations varied dramatically among varieties and environments (except desi environment). Hence no single trait had a positive effect on seed quality in all environments. Therefore it will not be feasible to improve quality by selecting for any specific trait in these varieties and environments.

3.4.4 Repeatability and heritability

Repeatability and heritability are two genetic and phenotypic parameters required for efficient planning of crop improvement programmes. Repeatability defines the correlation between measurements made on the same trait of the same plant or identical plant types over time or

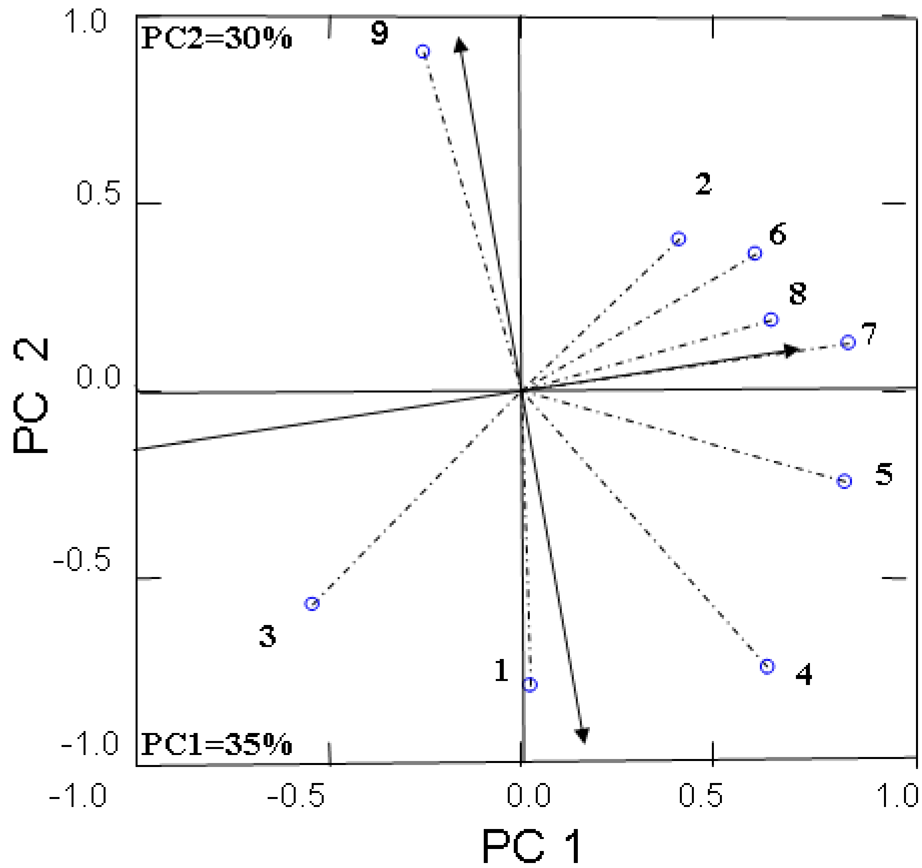

Figure 3.7 Plots of first two major principal components for genotypic effect on major seed components in kabuli chickpeas, representing genotypefocused, singular value partitioning for (a) amylose: 1, Amit; 2, CDC Frontier; 3, CDC Xena; 4, FLIP97-45C; 5, FLIP98-134C; 6, FLIP97-133C; 7, FLIP98-135C; 8, 97-Indian2-1; 9, Sanford.

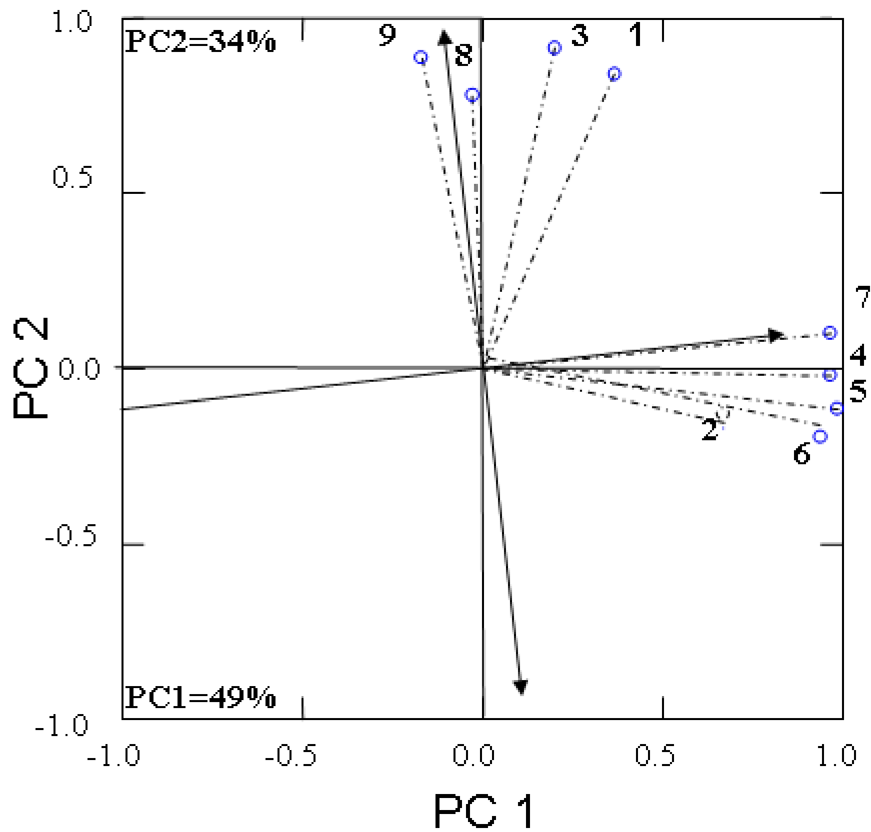

Figure 3.8 Plots of first two major principal components for genotypic effect on major seed components in kabuli chickpeas, representing genotypefocused, singular value partitioning for starch: 1, Amit; 2, CDC Frontier; 3, CDC Xena; 4, FLIP97-45C; 5, FLIP98-134C; 6, FLIP97-133C; 7, FLIP98-135C; 8, 97-Indian2-1; 9, Sanford.

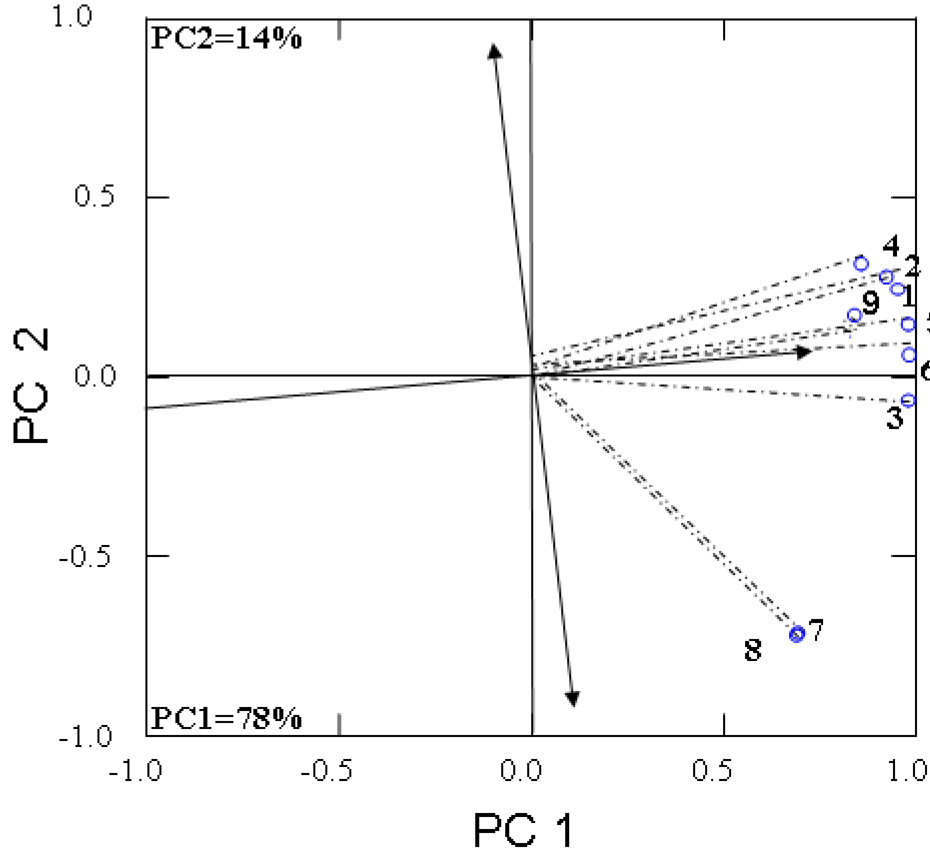

Figure 3.9 Plots of first two major principal components for genotypic effect on major seed components in kabuli chickpeas, representing genotypefocused, singular value partitioning for protein: 1, Amit; 2, CDC Frontier; 3, CDC Xena; 4, FLIP97-45C; 5, FLIP98-134C; 6, FLIP97-133C; 7, FLIP98-135C; 8, 97-Indian2-1; 9, Sanford.

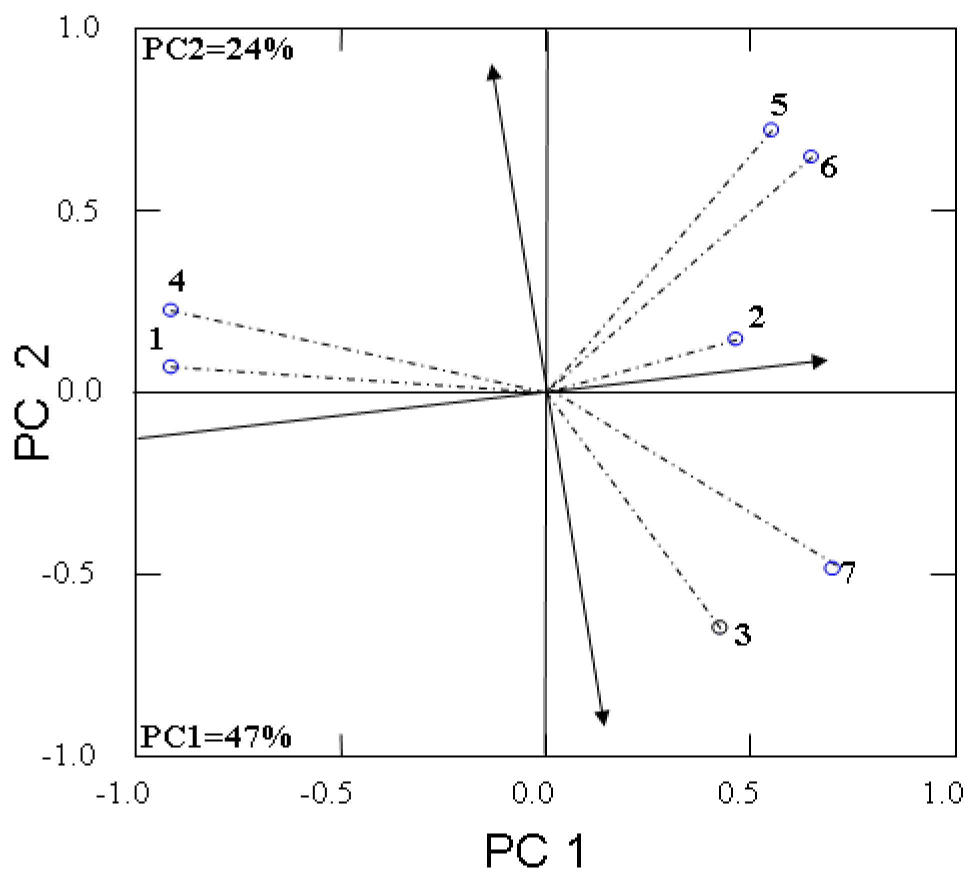

Figure 3.10 Plots of first two major principal components for environmental effect on major seed components in kabuli chickpeas, representing environment-focused, singular value partitioning for amylose: 1, Bow Island; 2, Davidson; 3, Elrose; 4, Goodale; 5, Hodgeville; 6, Kyle; 7, Swift Current.

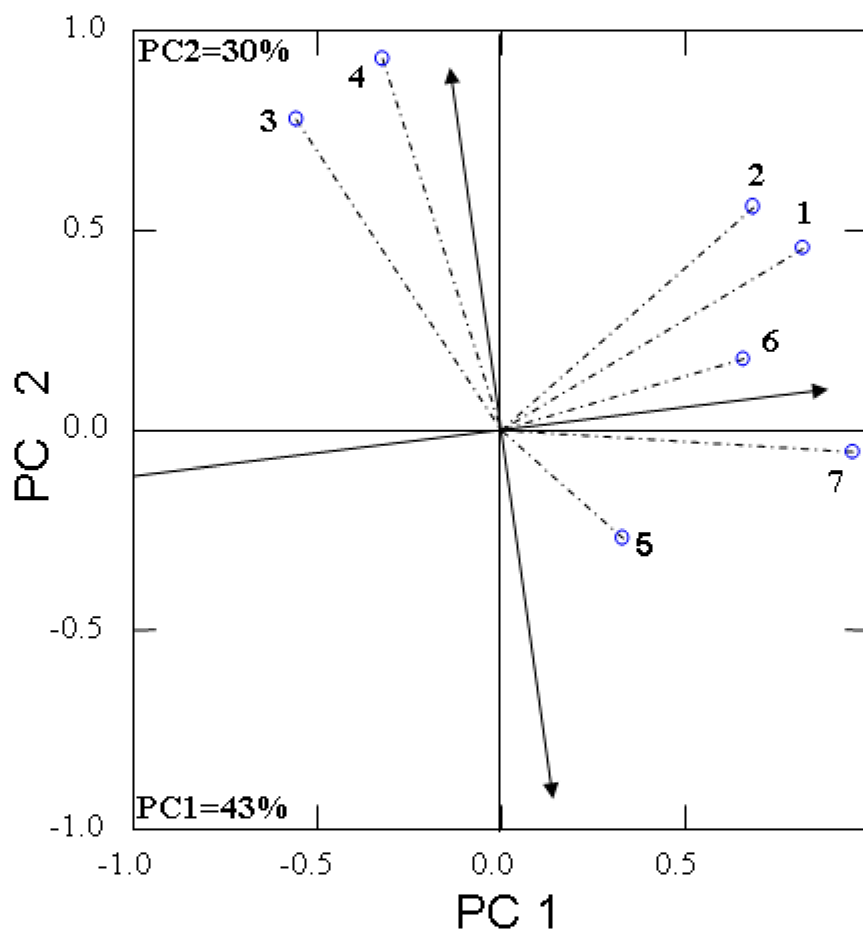

Figure 3.11 Plots of first two major principal components for environmental effect on major seed components in kabuli chickpeas, representing environment-focused, singular value partitioning for starch: 1, Bow Island; 2, Davidson; 3, Elrose; 4, Goodale; 5, Hodgeville; 6, Kyle; 7, Swift Current.

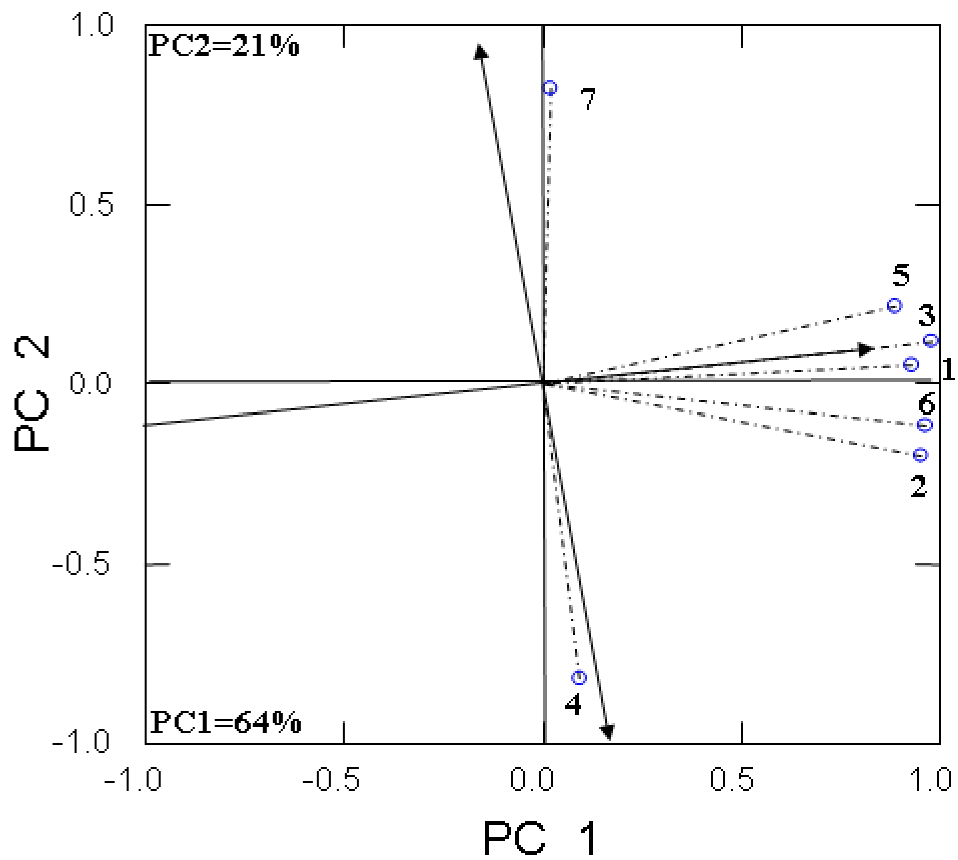

Figure 3.12 Plots of first two major principal components for environmental effect on major seed components in kabuli chickpeas, representing environment-focused, singular value partitioning for protein: 1, Bow Island; 2, Davidson; 3, Elrose; 4, Goodale; 5, Hodgeville; 6, Kyle; 7, Swift Current.

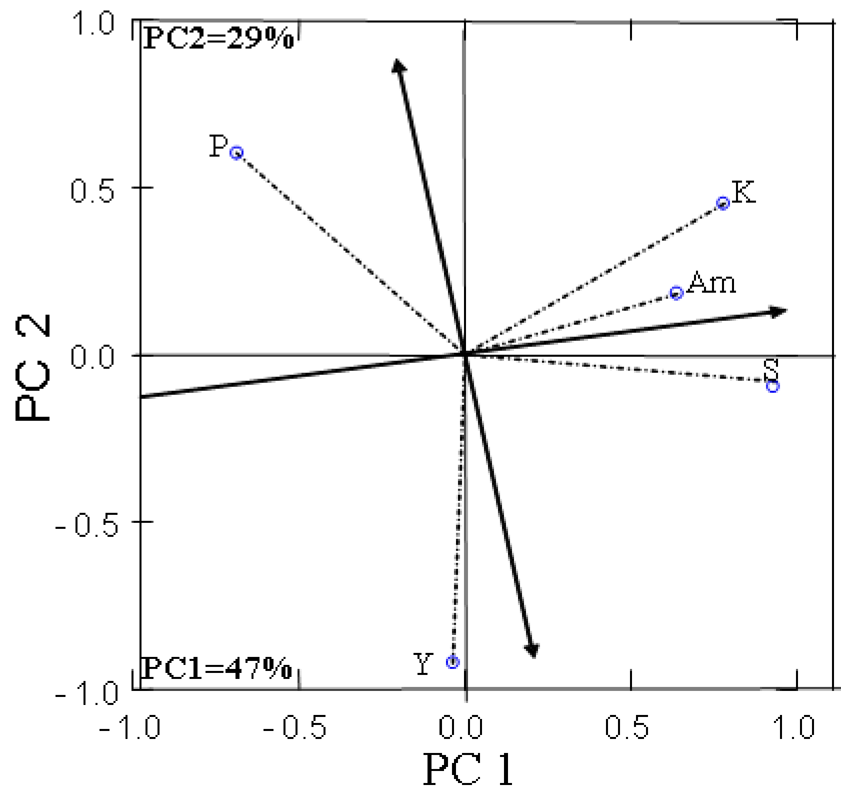

Figure 3.13 Trait-focused, singular value partitioning PCA plots indicating genetic correlations among five traits (S, starch; P, protein; Am, amylose; K, seed weight; Y, seed yield) in kabuli chickpea: genotype-focused, singular value partitioning for kabuli class.

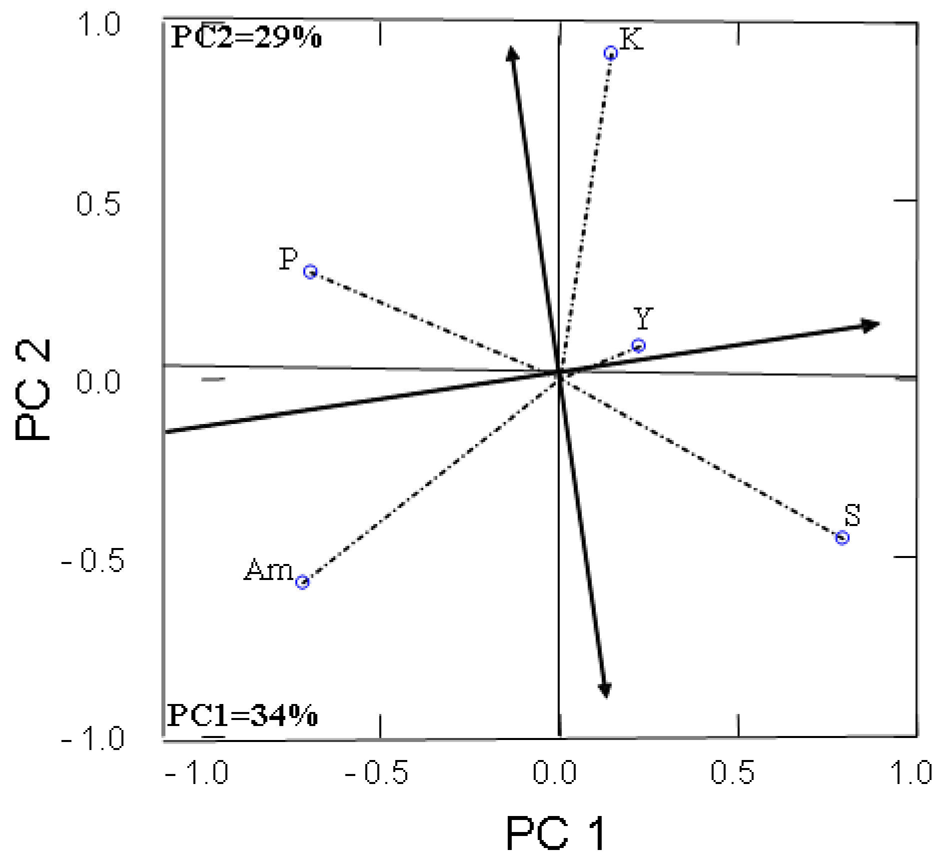

Figure 3.14 Trait-focused, singular value partitioning PCA plots indicating genetic correlations among five traits (S, starch; P, protein; Am, amylose; K, seed weight; Y, seed yield) in kabuli chickpea: environment-focused, singular value partitioning for kabuli class.

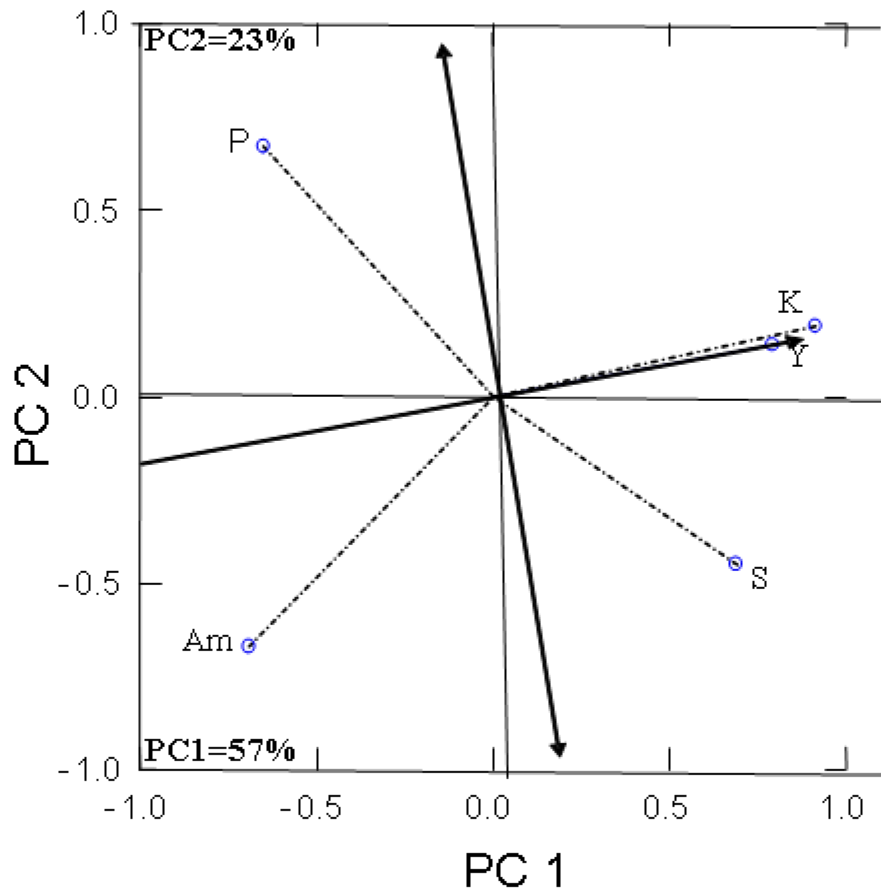

Figure 3.15 Trait-focused, singular value partitioning PCA plots indicating genetic correlations among five traits (S, starch; P, protein; Am, amylose; K, seed weight; Y, seed yield) in desi chickpea: genotype-focused, singular value partitioning for desi class.

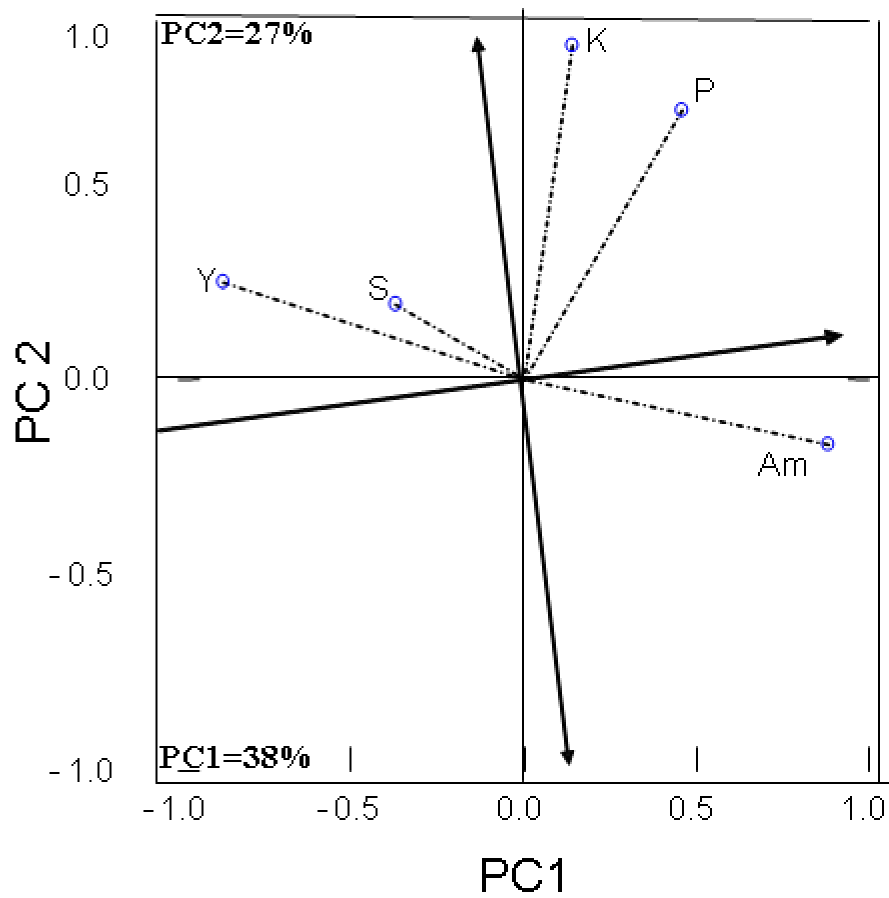

Figure 3.16 Trait-focused, singular value partitioning PCA plots indicating genetic correlations among five traits (S, starch; P, protein; Am, amylose; K, seed weight; Y, seed yield) in desi chickpea: environment-focused, singular value partitioning for desi class.

space (Roman et al. 2000). Heritability explains the extent to which observed differences between individuals are associated with additive genetic variance (variance of the breeding values) (Roman et al. 2000). Knowledge of both parameters can determine whether or not a particular trait can be improved by geneticists using selection, improvement of management practices or both. This report focuses on repeatability of quality traits. Seed yield and seed weight had repeatability values of 0.68 and 0.94 respectively for desi and 0.75 and 0.91 respectively for kabuli chickpeas (Table 3.7). Repeatability for protein concentration was higher (0.66) than that for starch concentration in desi but much lower (0.07) than that for starch concentration in kabuli chickpeas. However, amylose concentration had repeatability values of 0.44 and 0.18 for desi and kabuli chickpeas respectively. Single determination repeatability estimates of starch concentration were 0.12 and 0.49 for desi and kabuli chickpeas respectively. These were lower, but according to Hucl and Chibbar (1996) multiple determination of repeatability after additional testing increased repeatability. The small magnitude of the repeatability values of traits may possibly be due to the small sample size used and the narrow genetic base of the experimental materials. Although desi and kabuli chickpea market classes are of the same species, the inconsistencies in repeatabilities for protein, starch and amylose traits across market classes may be due to multiple genes in biosynthetic pathways that contribute to their synthesis (Chibbar and Baga, 2003; Morell and Myers, 2005).

Most of the starch biosynthetic genes are present in multiple forms, whose expression could be influenced by the environment. Failure of individual varieties to maintain the trait balance from one location to another and errors arising from extraction procedures could also account for low repeatability values. Dohm (2002) explained the former as lack of independence among the unique environmental effects for successive measurements.

Repeatability values of less than 1.0 could provide direction for additional tests. Our study was not of sufficient length to indicate stable, specific location adaptation, as demonstrated by low repeatability (Table 3.7). Low repeatability could also indicate practical problems associated with the measurement protocols. Repeatability measures genetic and environmental sources of variation, whereas heritability includes only genetic differences among individuals (Boake, 1989).

Table 3.7 Repeatability (r_{approx}) estimates for seed yield, seed weight and protein, starch and amylose concentrations of desi and kabuli chickpea varieties evaluated across environments in western Canada.

Trait	Desi r_{approx}	Kabuli r_{approx}
Seed yield	0.68	0.75
Seed weight	0.94	0.91
Protein concentration	0.66	0.07
Starch concentration	0.12	0.49
Amylose concentration	0.44	0.18

Table 3.8: Heritability ($H_{broad\ sense}$) estimates for seed yield, seed weight, protein, starch, and amylose concentrations of desi and kabuli chickpea varieties evaluated across environments in western Canada.

Trait	Desi $H_{broad\ sense}$	Kabuli $H_{broad\ sense}$
Seed yield	0.83	0.97
Seed weight	0.99	0.97
Protein concentration	0.93	0.59
Starch concentration	0.53	0.50
Amylose concentration	0.68	0.36

Heritability for the studied traits in these genotypes were higher than their respective repeatability values ranging between 0.53-0.99 in desi and 0.36-0.97 for kabuli (Table 3.8). This trend disagrees with Falconer and Mackey (1996) proposition that repeatability sets upper limits to heritabilities in braod sense. However, our results support Dohm (2002) proposal that repeatability estimates may not set the upper limits to heritability if significant genotype by environment interaction is present.

3.5 CONCLUSIONS

Genotype × environment interaction was significant ($P < 0.05$) for starch, amylose and protein (except for kabuli) concentrations, seed yield and seed weight. Selecting for high-amylose kabuli chickpea will mean selecting for low starch yield, whereas selecting for high-amylose desi chickpea will require selecting for high starch yield. The positive and significant relationships between starch concentration and seed yield in desi and between starch concentration and seed weight in kabuli offer breeders the possibility of indirect selection. The negative relationship between starch and protein concentrations across chickpea market classes will require a compromise during selection. Repeatabilities for starch, amylose and protein concentrations were low and inconsistent across chickpea varieties. However, like heritability, repeatability was genotype- and environment-specific and may be improved with additional testing environments to reduce error. However, the small magnitude of the correlation between traits and repeatability values may be due to the relatively small sample size utilised and the relatively narrow genetic base of the experimental materials. Among desi varieties, Myles and CDC Anna had the highest protein, starch and amylose concentrations, while Sanford and FLIP97-45C had the highest concentrations of protein, starch and amylose among kabuli varieties.Identifying the best strategies for selection of seed quality characteristics in chickpea improvement programmes will be affected by the size of the genotype × environment interaction for these traits. The negative relationship between some seed constituents and yield indicates that selection for chickpea cultivars with high seed quality may require compromise and indirect selection. However, progress can be made in improving various quality traits by using large segregating populations and relatively strict selection intensity. Alternatively, identification of mutants or development of molecular biological strategies could be used to develop varieties with desirable seed constituents.

CHAPTER 4

VARIATION IN CHICKPEA (*Cicer arietinum* L.) MINI CORE COLLECTION FOR SEED QUALITY TRAITS

4.1 ABSTRACT

Identification and utilization of genetic diversity is the basis for cultivar improvement. Genetic diversity investigations identify parents for hybridization programs tailored to achieve heterotic recombinants. The chickpea mini core collection was characterized for seed colour, seed diameter (size), thousand seed weight (TSW), starch, amylose, and protein concentration to identify accessions for potential use in chickpea seed quality improvement programs. In the desi accessions, mean seed diameter and TSW were 5.8±0.5 mm and 165±0.0g, respectively, while starch, amylose, and protein concentrations were 50.5±2.6%, 28.8±2.3%, and 25.9±2.0%, respectively. In kabuli accessions, seed diameter and TSW were 6.8±0.6 mm and 220.6±56.5 g, while starch, amylose and protein concentrations, were 47.3±2.6%, 27.4±2.2%, and 23.4±1.6%, respectively. Amylose concentration did not have any relationship with all other traits in both desi (except seed colour $r=0.67$, $p<0.05$) and kabuli (except seed diameter $r=0.47$, $p<0.05$). In desi and kabuli accessions, within trait correlation of amylose, protein, seed diameter and thousand seed weight (TSW) were significant ($p<0.001$). However, the assessed mini core subset did not exhibit significant quantitative variation for intrinsic seed quality traits, despite a high Shannon-Weaver Diversity index. To obtain a larger amount of variation in the intrinsic seed quality traits, a new and larger-sized mini core collection based on expanded collections to enrich the diversity in current collected chickpea accessions needs to be screened.

4.2 INTRODUCTION

Chickpea is grown across the major agro ecological zones of the world over an area of 8.8 million hectares in 51 countries with an annual production of over 11.6 million metric tonnes (FAO, 2009). Two chickpea market classes are recognized, the dark seed coat colour, small seeded, angular and fibrous desi type and the beige, large seeded, rams-head shaped and lower fiber kabuli type. Chickpea grain quality can be extrinsic (seed colour, seed size and seed diameter) or intrinsic (starch, amylose, protein and other nutrient contents). The seed is

consumed for its high protein (25 - 29%) and starch (30 - 57 %) concentration as well as other essential human nutrients. Approximately 20 - 47 % of chickpea starch is amylose (Wood and Grusak, 2007). Starch plays an important role as the dominant carbohydrate in human diets and serves as the main carbon reserve in many grain legumes including chickpea. The content and composition of carbohydrate in chickpea seed may also affect the physical attributes of the grain and its use in human diets (Hedley, 2001). In pea (*Pisum sativum),* the genetic variation for seed starch content and its composition has been well characterized (Wang et al., 1998). More than 30 starch mutants have been characterized in pea (Wang et al., 2003). These mutations alter the seed shape from round to wrinkled, cause changes in starch and amylose content, amylopectin architecture, and starch granule composition and structure (Wang et al., 2003). As compared to cereal grains, legume grains have a higher amylose concentration in their starches making them less bioavailable either raw or retrograded (Guillon and Champ, 2002). Lower bioavailability of starches reduces their glycemic index, makes legumes starches such as chickpea, beneficial for prevention of insulin resistance related diseases.

Seed storage proteins in legumes have been classified as either albumin or globulin types, depending on their solubility in water or dilute salt solution (Croy and Gatehouse, 1985). Genetic diversity for the quantity and quality of legume seed proteins has been investigated in many genera including *Pisum* (Schroeder, 1982), *Arachis* (Bertozo and Valls, 2001) and *Vicia* (Potokina and Eggi, 1997), and alfalfa *(Medicago sativa* L.; Krochko and Bewley, 2000). However limited information exists on the genetic diversity for extrinsic and intrinsic seed quality traits in chickpea germplasm.

The Consultative Group on International Agricultural Research (CGIAR) supported two Institutes, International Crops Research Institute for the Semi-Arid Topics (ICRISAT), India and International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria to maintain the global chickpea germplasm collections. ICRISAT has 17,258 accessions (135 wild and 17,123 cultivated) and ICARDA maintains 12,647 accessions (304 wild, and 12,343 cultivated) of chickpea representing most of the global germplasm (Upadhyaya et al., 2008). Inspite of the large number of genetic resources available, only a very limited number of improved chickpea varieties have been developed using core collections. To facilitate the utilization of available germplasm collections, the concept of core collection was developed (Brown, 1989a). A core collection is a subset of accessions from the entire collection that captures most of the genetic

diversity available in the gene pool in a gene bank. A major consideration in setting a core subset is the size. Using the sampling theory of selectively neutral alleles, Brown (1989a) suggested that the entries in a core set should be at least 10% of the total accessions, with a top limit of 3,000 accessions. Upadhyaya et al. (2001) developed a core subset of 1,956 chickpea accessions based on the geographic distribution and 13 quantitative traits. The 13 quantitative traits used to develop the core collection, included: days to 50% flowering, plant height, plant width, days to maturity, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, tertiary branches, pods per plant, seeds per pod, seed yield, and 100-seed weight. The core collection with 1956 genotypes is also very large to assess traits related to seed quality such as seed composition, which often show genotype x environment interaction (chapter 3). Upadhyaya and Oritz (2001) developed a two stage strategy to select a chickpea mini-core subset consisting of only 1% of the entire collection at ICRISAT. In the first step a core collection was developed using the geographical origin, distribution, and characterization and evaluation data available in the gene bank. In the second stage the core subset was evaluated for various morphological, agronomic and quality traits to select 10% of the core accessions. At both stages standard clustering procedures were used. A mini core subset of 211 chickpea accessions was developed (Upadhyaya and Oritz, 2001). To date, the chickpea mini core collections have been characterized for 22 morphological and agronomic traits (Upadhyaya and Ortiz, 2001), disease resistance (Pande et. al., 2005) and drought tolerance (Serraj et al., 2004). The main objective of this experiment was to use the chickpea mini-core collection to assess natural variation in extrinsic seed quality traits and seed composition when grown in Saskatchewan conditions.

4.3 MATERIALS AND METHODS

4.3.1 Plant Materials

A total of 214 accessions, including 209 accessions comprising the chickpea mini core collection (ICC 15406 from Morrocco and ICC16796 from Portugal were not used in this study) developed by Upadhyaya and Ortiz (2001) were obtained from the International Crops Research Institute for Semi-arid Tropics, Patancheru, Andhra Pradesh, India. In addition, a desi accession (ICC 4948) and four Kabuli accession (ICC 4973, ICC12968, ICC 3162 and ICC 3279) all from India were also included in the trials. The entries were divided into two major groups (market classes):

(1) desi with purple flower, angular seed shape, light tan to tan seed coat colour and thick hull (162 entries), and (2) kabuli with white flower, thin hull and beige seed colour (52 entries). All the 214 lines were seeded in 2006. In 2007 only 143 accessions (41 kabuli and 102 desi) were grown due to lack of seed supply for those accessions that were highly susceptible to *Ascochyta blight* or of very late maturity. In addition, six Desi (CDC Anna, CDC Cabri, CDC Desiaray, Myles, and lines 304-22 and ICC 12512-1) and six Kabuli (Amit, FLIP97-133C, FLIP97-135C, CDC Chico, CDC Chi Chi and CDC Frontier) were also grown at the same locations in both the years. Samples of each genotype and each year were analyzed independently. Triplicate measurements were done on each sample for seed coat colour, total starch, amylose concentration and total protein.

4.3.2 Growing sites and conditions

The accessions were grown in an unreplicated trial at Kyle, Saskatchewan, Canada (50°49'N - 108°1'W) in the summer of 2006 and 2007. Seeding was done on May 10 in 2006 and on May 22 in 2007. The site belongs to the Chernozemic Order with 1-17% organic carbon, C/N ratio of less than 17 and dominant in Ca (Pennock, 2006). Kyle is located within the Brown Soil Zone of western Canada. The mean temperature (May-August) in 2006 and 2007 was16.3^{o}C and 15.4 ^{o}C, respectively, with total precipitation (May-August) of 215 mm and 234 mm in 2006 and 2007, respectively. Plot size was 1 m^{2} with 30 cm distance between adjacent plots. Fifty seeds were sown in three short rows for each plot. Seeds were treated with fungicide Apron FL® before planting to protect from soil-borne pathogens. Crop maintenance in field followed the standard cultural practices for the area: hand weeding and controlling ascochyta blight (*Ascochyta rabiei*) with an application of prothioconazole (Proline 480 SC) at 150 mL per acre.

4.3.3. Extrinsic characters

4.3.3.1 Thousand-seed weight (g)

Seed weight was measured on 250 seeds at 12% moisture content. The value was then converted to thousand seed weight (TSW).

4.3.3.2 Seed diameter (mm)

Seed diameter was measured as the mean of maximum trans-section of 50 seeds for each accession using a digital vernier caliper.

4.3.3.3 Seed coat colour

The seed coat colour was measured using a Hunter colourimeter in the *L*, *a**, *b** scale (Colour Quest XE Hunter Lab, VA, USA). The colour measurement was performed three times and an average value was used in the analysis.

4.3.4 Intrinsic quality determinations

4.3.4.1 Starch concentration

The desi seeds were dehulled before milling, whereas the kabuli seeds were milled intact prior to the analysis. Total starch was determined using the Megazyme (AA/AMG) method (McCleary et al., 1994) as described in section 3.3.4.

4.3.4.2 Amylose concentration

Amylose was determined using a high performance size-exclusion chromatography (HP-SEC) method (Demeke et al., 1999). Five milligrams of starch sample was debranched as described and used for amylose concentration determination in section 3.3.6.

4.3.4.3 Protein concentration

Total seed protein was estimated by multiplying the sample's nitrogen content (N_2) with a factor of 6.25 (AACC, 2000) as described in section 3.3.7.

4.3.5 Data analysis

Means and ranges of each quality characteristic and year were calculated. Correlations between 2006 and 2007 data for each trait as well as correlations among all the mean traits over the two years were computed using the SAS software, version 9.1(SAS Institute, Cary, North Caroline, USA). Phenotypic diversity was estimated (Bhattacharjee et al. 2007) by the Shannon-Weaver diversity index (SDI) (Shannon and Weaver 1949) as follows:

$$\mathrm{SDI} = (-\Sigma^{n}_{i=1} P_i \times \log_e P_i)/\log_e n$$

Where, n = number of phenotypic classes for a trait,

Pi = proportion of the total number of entries in the ith class.

The phenotypic data was grouped into classes using the Minitab software version 16 and number of genotypes in each class was determined. The SDI was calculated in Excel using the formula described above.

4.4 RESULTS AND DISCUSSIONS

4.4.1 Mini-core collection

In this study, a chickpea germplasm collection comprising of 214 genotypes, with 162 desi, 52 Kabuli genotypes was evaluated over two years at the same site. Two desi and four kabuli genotypes were from the centre of origin, while 42 desi and 21 kabuli genotypes were from the primary centre of diversity (Figure 4.1, Table 4.1). 90 desi and 10 kabuli genotypes represent a major secondary centre of diversity comprising of Bangladesh, India, Myanmar, Nepal and Pakistan. The other regions contributed only a few genotypes of each class, except Ethiopia from where 13 desi genotypes were collected (Table 4.1).

In the first year (2006) of the study, seeds were obtained from all the genotypes planted and observations were recorded for all the traits of interest. However, in the second year of study (2007), a severe Ascochyta infection occurred and adequate amount of seeds could only be obtained from 64 desi and 21 kabuli genotypes for which data on all six traits could be recorded.

4.4.2 Extrinsic characters – seed colour, shape and size

The seed colour was measured using the L*, a*, b* method which measures the whiteness or darkness of seed coat. The higher L value shows that seed is whiter or creamier in colour. The L value ranged from 17.5 in dark-seeded ICC6293 of Italian origin to 56.3 in ICC11284, beige-seeded of Russian origin with an overall two-year mean of 29.6±8.7. The seed colour of desi genotypes was mostly brown or dark brown (90%) and black (9%) with a pink (ICC4872) and green (ICC5383) coloured accession from India. One quarter of accessions from Iran were black, while both accessions from Italy had black coloured seeds (Figure 4.2). Pakistan and India had one each and Ethiopia had two accessions with black coloured seeds. All kabuli accessions had beige coloured seeds. Chickpea seeds were mostly of two types: desi types had angular seeds, whereas kabuli types had mostly owl head shaped seeds. Some of the kabuli types also had pea-shaped seeds (Figure 4.3).

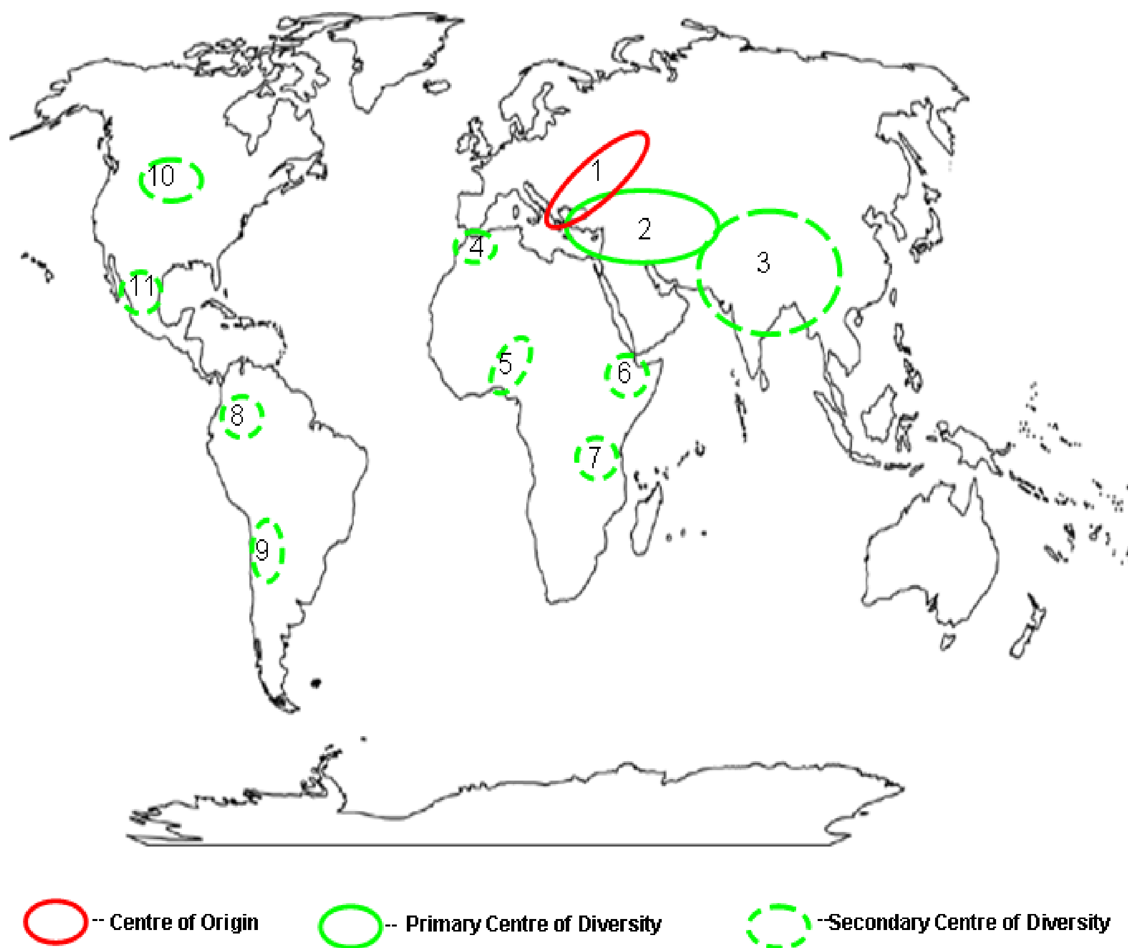

Figure 4. 1. Centers of origin and diversity for chickpea (*Cicer arietinum* L.).

Table 4.1. Representation of chickpea mini core (number of accessions) by region and type as shown in Figure 4.1.

Region	Countries	Number of accessions	
		Desi	Kabuli
1	Russia, Turkey	4	8
2	Afghanistan, Cyprus, Iran, Israel, Syria	40	17
3	Bangladesh, India, ICRISAT (India), Myanmar, Nepal, Pakistan	90	10
4	Algeria, Morocco	2	5
5	Nigeria	1	0
6	Ethiopia	13	1
7	Malawi, Tanzania	5	0
8	Peru	0	1
9	Chile	1	0
10	USA	0	1
11	Mexico	3	1
Total		159	44

Fig. 4.2. Variations of colour in chickpea mini core collection.

Fig. 4.3. Variations of seed shape in chickpea mini core collection.

In 2006, desi seed diameter ranged between 5 and 8 mm with mean of 5.8±0.5 mm (Table 4.2). Seed diameter ranged from 4-7 mm with mean of 5.5±0.7 mm in 2007. These values compare favorably with the local checks of 6-8 mm (mean 6.9 mm) and 6-7 mm (mean 6.0 mm) for 2006 and 2007, respectively. The Canadian varieties had larger seed sizes (6.85 mm) with CDC Cabri with the largest seed diameter (7.49 mm). A Russian accession ICC6306 with a seed diameter (7.30 mm) was the largest seed size among the 162 accessions studied. Indian accessions had the largest variation in seed diameter (4.8 to 7.0 mm). Compared to the desi types, kabuli chickpea accessions had larger seed sizes with an average diameter ranging between 6 to 8 mm (Table 4.2) The Canadian varieties had larger seeds, with both FLIP97-133C and FLIP97-135C recording largest seed sizes (diameter 8.2 mm). However, the variation in chickpea kabuli genotypes was less pronounced as compared to desi-types. This classifies desi chickpea mini core into medium to large seed size (Upadhyaya et al. 2006b). The differences in mean seed size for kabuli and desi could be attributed to differences in cotyledon cell numbers (Munier-Jolain and Ney, 1995). Seed size affects crop survival and adaptation, yield and consumer preference.

The desi genotypes showed more than two-fold variation in TSW, with Canadian varieties showing the highest TSW (340 g) (Table 4.2). As in seed size, most variation in TSW was also found in Indian accessions, in which ICC1882 had the least TSW (109 g) while ICC1915 had the highest TSW (283 g). The Russian accession ICC6306 with the largest seed diameter also had high TSW (268 g). The kabuli chickpea accessions had higher TSW as compared to desi chickpea accessions. A Canadian genotype FLIP97-133C had the highest TSW (420 g). Variation in seed weight of entire chickpea collection at ICRISAT had been reported to range from 40-630 mg $seed^{-1}$ (Upadhyaya, 2003) with over 60% weighing 90-140 mg $seed^{-1}$. Seed size had been reported (Upadhyaya, 2006b) to be under epistatic interaction/inheritance hence a cross between small and medium-large may give a recombinant with better seed size. Seed size variation could be attributed to geographical pattern and/or different fitness components of seedlings and adult plants (Narayanan et al., 1981, Dahiya et al., 1985).

Table 4.2. Mean/range and standard deviation for seed diameter, seed weight and colour measurement of the chickpea mini core collection in 2006 and 2007 evaluated at Kyle, SK, Canada.

		Number of accessions	Seed diameter (mm)	Thousand Seed weight (g)	Colour		
					L*	a*	b*
Year	Type/Class						
2006	Desi	162	5.8±0.5 5-8	160.0±39 113-320	31.7±7.4 17-56	6.9±2.7 -0.7-12	11.3±4.1 1.4-18
	Local checks (desi)	6	6.9±0.5 6-8	250.0±50 200-340	34.9±2.7 31-39	10.4±1.0 9-11	13.6±0.8 13-14
	Kabuli	52	7.1±0.5 6-8	249.0±52 181-341	48.1±5.2 28-56	8.2±1.1 4-10	15.7±1.6 8-19
	Local checks (kabuli)	6	7.9±0.5 7-8	367.0±66 273-436	51.8±1.2 50-54	8.2±0.5 7-9	15.9±0.7 15-16
2007	Desi	64**	5.6±0.6 4.3-7.2	153.8±35.2 115.6-272.9	27.1±8.7 16.2-57.0	5.2±2.9 0.5-9.0	8.2±4.6 0.6-16.5
	Local checks (desi)	6	6.0±0.3 6-7	218.0±38 178-279	31.9±3.8 28-39	10.1±1.0 8-11	11.9±2.1 10-16
	Kabuli	21***	6.6±0.7 5.3-7.9	201.9±72.2 77.1-315.8	45.7±7.7 37.0-55.2	7.3±1.6 1.9-9.4	14.7±3.0 2.7-16.9
	Local checks (kabuli)	6	7.4±0.6 7-8	317.0±65 231-409	52.27±1.3 51-54	8.3±0.4 7-9	16.1±0.3 15-16
2006 + 2007	Desi	64	5.8±0.52 4.8-7.3	165.0±0.0 117.9-283.0	29.6±8.7 16.7-46.0	5.7±2.9 0.5-10.2	9.5±4.7 0.8-15.8
2006 + 2007	Kabuli	21	6.8±0.6 6.1-7.9	220.6±56.5 137.4-365.0	47.6±5.3 33.0-55.8	7.8±1.1 5.3-9.2	15.4±1.7 9.4-17.5

**Total of 120 accessions had data recorded on them but 64 had data on all the six quality traits due to *Aschochyta* incidence.
***Total of 42 accessions had data recorded on them but 21 had data on all the six quality traits due to *Aschochyta* incidence.

4.4.3 Intrinsic seed characteristics – Starch, amylose and protein concentration

Starch and protein are the two major constituents of chickpea seeds (Chibbar et al 2010). Amylose is a starch component that affects starch hydrolytic properties and its end-use for food, feed and industrial applications (Chibbar et al. 2007). The desi and kabuli-type chickpea accessions had similar starch concentrations (Table 4.3). In desi-type chickpea the hull was removed prior to grinding. This could be the reason for slightly higher starch concentrations in desi type chickpea genotypes. A desi chickpea accession ICC6877 from Iran had the highest starch concentration (56.48 %), while an Indian accession ICC1180 had the lowest starch concentration (43.58 %). In the kabuli type chickpea, Iranian accessions showed the most variation with ICC9402 (41.1%) the lowest and ICC15264 the highest (55.4%) starch concentrations. The Canadian varieties had an average of 49.4% starch concentration with FLIP97-133C seeds with a starch concentration of 53 %.

The average amylose concentration in both the desi and kabuli genotypes at 27- 28% did not vary between the two chickpea types or in Canadian genotypes (Table 4.3). However, both the desi (22 – 36 %) and kabuli (23 – 35 %) accessions had a wider range of amylose concentration than Canadian genotypes (27 – 30%) (Table 4.3). The desi chickpea accessions from India showed the most variation but the ICC2884 from Iran had the highest (32.7%) and another ICC27554, had the lowest (24.5%) amylose concentration.

In the kabuli type chickpea, ICC2277 from Iran had the highest (31.75%), while ICC8151 from USA had the lowest (21.6%) amylose concentration. The mean amylose concentration observed in this study is less than 32%, which is lower than most of the chickpea amylose concentrations reported in the literature (Hoover et al 2010). Amylose concentrations reported in this study were determined by HPLC analysis of debranched starch (Demeke et al 1999), which is much more precise than other methods reported in the literature (Chibbar et al 2010; Hoover et al 2010).

The desi type chickpea accessions showed a higher range (19 to 30 %) as compared to kabuli type chickpea (21 to 27 %) of protein concentration (Table 4.3). In both types, the Canadian genotypes showed slightly lower protein concentration. There was less variation for protein concentration, but for desi type chickpea an Iranian accession ICC15294 had the lowest (19.3 %) and a Mexican accession (ICC12307) had the highest (30.4 %) protein concentration.

Table 4.3. Mean/range and standard deviation for grain starch, amylose and protein concentration of chickpea mini core collection in 2006 and 2007 evaluated at Kyle, SK, Canada.

Year	Type/Class	Number of accessions	Total starch (%)	Amylose	Total protein (%)
2006	Desi	162	51.4±3.6 42-61	27.6±1.2 24-32	25.1±2.2 19-31
	Local checks (desi)	6	54.2±2.9 51-58	28.9±1.0 27-30	22.4±2.1 20-26
	Kabuli	52	49.4±3.6 41-55	27.4±1.3 24-30	21.4±1.7 18-26
	Local checks (kabuli)	6	53.3±1.4 51-55	28.5±0.7 27-30	21.4±1.3 20-23
2007	Desi	64**	48.6±3.4 43.4-56.2	29.9±4.1 24.5-36.0	27.5±2.3 18.0-32.8
	Local checks (desi)	6	50.6±1.9 48-52	27.1±0.8 26-28	26.8±1.6 25-29
	Kabuli	21***	44.0±3.5 38.3-52.3	27.5±3.6 22.6-35.3	25.1±1.6 23.3-29.2
	Local checks (kabuli)	6	44.3±3.0 39-48	27.7±0.6 27-28	24.4±1.2 23-26
2006 + 2007	Desi	64	50.5±2.6 44.8-55.8	28.8±2.3 24.3-32.7	25.9±2.0 19.3-29.8
2006 + 2007	Kabuli	21	47.3±2.6 43.6-52.1	27.4±2.2 24.4-31.7	23.4±1.6 21.6-27.1

**Total of 120 accessions had data recorded on them but 64 had data on all the six quality traits due to *Aschochyta* incidence.
***Total of 42 accessions had data recorded on them but 21 had data on all the six quality traits due to *Aschochyta* incidence.

In kabuli type chickpea, ICC15697 from Syria had the lowest (18.2) and ICC9862 and ICC9895 both from Afghanistan had the highest (27.1 %) protein concentration.

4.4.4. Trait correlations

Statistical analysis revealed a significant difference ($P \leq 0.001$) when means of Kyle 2006 and Kyle 2007 TSW (160.0g, 154.0g), L (31.7, 27.1), a (6.9, 5.2), and b (11.3, 8.2) values were respectively compared using t-test. Total starch (51%, 49%), amylose (27.6%, 29.9%) and protein (25%, 27.5%) followed a similar trend (Table 4.4). Desi amylose and protein means of 28.8±2.3% and 25.9±2.0% agree with Saini and Knights' (1984) and Viveros et al. (2001) observations. However considering that these values are records mostly from only one location (Kyle) although in different years (2006 and 2007), it implies reliable values for amylose and protein traits in desi will only be evident after accessions are tested multi locationally. Desi total starch mean across the two years however, was 50.5±2.6%. This is higher compared with Saini and Knights' (1984) observations. In both market classes, accessions having extreme starch contents had average protein contents.

Amylose did not have any relationships with seed diameter, thousand seed weight, starch and protein in desi (Table 4.4). However, it had a significant correlation with seed colour ($r=0.67$, $p<0.01$). This implies the brown-coloured seed desi types had more amylose. Meanwhile TSW significantly correlated ($r=30$, $p<0.01$) with total starch in desi.

Protein negatively and significantly correlated with seed diameter, TSW, and starch (except kabuli) in both desi and kabuli (Tables 4.4 and 4.5). This implies in both chickpea class, progress in breeding for protein, seed diameter, starch and TSW combined will be slowed by the strong negative correlations between protein and these traits. Starch in desi correlated positively with seed diameter ($r=0.31$, $p<0.01$) and thousand seed weight ($r=0.30$, $p<0.01$). No relationship was observed between starch and seed coat colour in desi. Out of the traits tested, only total starch did not have significant within-trait correlation when Kyle 2006 and Kyle 2007 data were compared in both market classes implying a lot of resources would be required in improving such a trait. Starch did not have any relationship with seed diameter, TSW, amylose, protein and L* in kabuli (Table 4.5). Amylose significantly correlated ($r=0.47$, 0.05) with seed size implying larger seed-sized kabuli had higher amylose contents. The smallness of this correlation coefficient may be attributable to the relatively smaller size of kabuli accessions in the mini core. Amylose had no relationship with TSW, protein, and seed coat

Table 4.4: Correlation coefficients among six quality traits in desi mini core of 2006 and 2007 growing season in Kyle, Canada.

Trait	Seed diameter (mm)	TSW (g)	Starch (%)	Amylose (%)	Protein (%)	L∞
Seed diameter (mm)	*(0.51**)*					
TSW (g)	0.88**	*(0.68**)*				
Starch (%)	0.31**	0.30**	*(0.05ns)*			
Amylose (%)	0.07ns	0.10ns	-0.11ns	*(0.30*)*		
Protein (%)	-0.51**	-0.52**	-0.57**	-0.04ns	*(0.56**)*	
L∞	0.17ns	0.14ns	-0.04ns	0.67**	-0.12ns	*(0.78**)*

** and * are significant at 1% and 5% probability level, respectively. ns means not significant. Values in italics and parenthesis are correlation within traits but between 2006 and 2007 samples at Kyle. N=64.
∞ denotes whiteness/blackness of seed coat using the L*, a*, b* method. Higher L means more white.

Table 4.5: Correlation coefficients among six quality traits in kabuli mini core of 2006 and 2007 growing season in Kyle, Canada.

Trait	Seed diameter (mm)	TSW (g)	Starch (%)	Amylose (%)	Protein (%)	L∞
Seed diameter (mm)	*(0.58**)*					
TSW (g)	0.92**	*(0.75**)*				
Starch (%)	0.35ns	0.34ns	*(0.12ns)*			
Amylose (%)	0.47*	0.29ns	-0.06ns	*(0.56**)*		
Protein (%)	-0.51*	-0.50*	-0.15ns	-0.34ns	*(0.62**)*	
L∞	0.08ns	0.07ns	-0.016ns	0.43ns	*-0.43**	*(0.56*)*

** and * are significant at 1% and 5% probability level, respectively. ns means not significant. Values in italics and parenthesis are correlation within traits but between 2006 and 2007 samples at Kyle. N=21.
∞ denotes whiteness/blackness of seed coat using the L*, a*, b* method. Higher L means more white.

colour. Protein negatively correlated with seed coat colour ($r = -0.43$, $p>0.05$) in kabuli.

4.4.5 Shannon-Weaver Diversity (SDI) index

The objective of a minicore collection is to provide a manageable size of germplasm collection which preserves the phenotypic variation and the genetic diversity available in the entire collection. Upadhyaya and Oritz (2001) compared the Shannon-Weaver diversity index (SDI) for 22 morphological traits in the core and mini core subsets of the chickpea and found good agreement between the two collections. Using a similar strategy, we calculated the SDI for the seed extrinsic and selected intrinsic properties analyzed in this study. For calculating the SDI, the traits were grouped into classes using Minitab (version 16). The number of genotypes in each class was entered into an Excel spreadsheet to calculate the SDI (section 4.3.5). The seed weight and seed colour were two traits which were common between the previous study (Upadhyaya and Oritz, 2001) which developed the mini core and this study. We calculated the SDI for the kabuli and desi chickpea genotypes and for the two years, separately due to the large variation in number of genotypes surviving in each year. Mean of the genotypes common to both years were also used for a combined SDI calculation for 2006 and 2007.

The SDI for desi chickpea seed colour determined as three separate parameters, L*, a* and b* separately was calculated to be between 0.811 – 0.861, 0.838 – 0.923, and 0.837 – 0.889 for 2006, 2007 and both years combined (Table 4.6). Similarly for kabuli type chickpea, the L*, a* and b* values were from 0.688 – 0.564, 0.707 – 0.433 and 0.815 – 0.714 for the years 2006, 2007 and both years, together, respectively (Table 4.7). Upadhyaya and Oritz (2001) did not separate the mini core collection into desi and kabuli as done in this study. Nevertheless, the SDI obtained in this study is very similar to that obtained for the core (0.856) and the mini core (0.871) collection (Upadhyaya and Oritz, 2001) used in this study. For seed weight, the SDI was 0.505 and 0.493 for the core and mini core collection, but for both desi and kabuli chickpea accessions, SDI between 0.759 to 0.791 and 0.812 to 0.929, respectively were calculated (Tables 4.6 and 4.7).

Seed weight is very strongly influenced by growing conditions. Most of the accessions were from the Indian subcontinent and middle-eastern region, thus Saskatchewan conditions may not be optimal for all the genotypes. Similarly, a high SDI (≥ 0.75) was observed for all the seed composition traits such as starch, amylose and protein concentration and seed size. The high SDI is expected as most of the accessions are from areas in the Indian subcontinent and middle-

Table 4.6 Shannon-Weaver Diversity Index (SDI) for chick pea mini core (desi genotypes).

Trait	Year		
	2006	2007	2006 + 2007
TSW	0.759	0.766	0.791
Seed Size	0.793	0.893	0.858
Starch	0.882	0.862	0.903
Amylose	0.802	0.805	0.866
Protein	0.849	0.734	0.800
L^*	0.829	0.838	0.837
a^*	0.811	0.904	0.882
b^*	0.861	0.923	0.889

Table 4.7 Shannon-Weaver Diversity Index for chickpea mini core collection (kabuli genotypes).

Trait	Year		
	2006	2007	2006 + 2007
TSW	0.929	0.812	0.836
Seed Size	0.857	0.939	0.900
Starch	0.885	0.905	0.933
Amylose	0.872	0.753	0.780
Protein	0.875	0.776	0.775
L^*	0.688	0.707	0.815
a^*	0.729	0.702	0.868
b^*	0.564	0.433	0.714

Table 4.8 Comparison of Shannon-Weaver Diversity index at the primary (region 2) and secondary (region 3) centres of chickpea diversity (desi genotypes).

Trait	Region – 2 – Primary centre of diversity Year			Region -3 – Secondary centre of diversity Year		
	2006	2007	2006+2007	2006	2007	2006+2007
TSW	0.857	0.926	0.787	0.670	0.794	0.798
Seed size	0.871	0.758	0.821	0.740	0.942	0.905
Starch	0.899	0.896	0.919	0.886	0.909	0.815
Amylose	0.873	0.826	0.757	0.923	0.689	0.876
Protein	0.839	0.671	0.749	0.945	0.883	0.918
L^*	0.961	0.890	0.950	0.755	0.804	0.818
a^*	0.852	0.822	0.809	0.752	0.804	0.829
b^*	0.866	0.900	0.863	0.774	0.877	0.764

Table 4.9 Comparison of Shannon-Weaver Diversity index at the primary (region 2) and secondary (region 3) centres of chickpea diversity (kabuli genotypes).

Trait	Region – 2 – Primary centre of diversity Year			Region -3 – Secondary centre of diversity Year		
	2006	2007	2006+2007	2006	2007	2006+2007
TSW	0.901	0.702	0.766	0.785	0.5804	0.712
Seed size	0.944	0.912	0.818	0.942	0.534	0.773
Starch	0.915	0.804	0.859	0.930	0.712	0.500
Amylose	0.871	0.637	0.701	0.915	0.646	0.430
Protein	0.765	0.778	0.766	0.742	0.534	0.646
L^*	0.697	0.887	0.782	0.638	0.773	0.773
a^*	0.810	0.887	0.804	0.660	0.773	0.861
b^*	0.578	0.887	0.804	0.315	0.861	0.646

eastern region, which when grown in exotic conditions, the genotype by environment affect gets more accentuated.

4.4.6 – Comparison of the diversity in primary and secondary centres of diversity

Most of the accessions originated from two regions (Table 4.1), the primary (Region 2) and secondary (Region 3) cenrtres of diversity. SDI was calculated separately for the two regions to determine if genotypes from either of the regions preferentially contributed to the high diversity observed for these traits. Both the primary and secondary centres of diversity did not differ in their SDI for the desi chickpea genotypes. The SDI were similar for both the centres of diversity (Table 4.8). For protein and amylose concentration and seed size, the SDI is slightly higher for the primary centre of diversity than the secondary centre of diversity.

Kabuli genotypes also have a very similar SDI for both the regions, except that the primary centre of diversity shows slightly higher SDI for all the traits studied (Table 4.9). The comparatively low SDI for starch (0.5) and amylose (0.43) concentration may be due to the very limited number of genotypes available for study in the secondary centre of diversity.

The results show a considerable diversity for the seed extrinsic and intrinsic properties analyzed in this study. SDI is used to measure both allelic richness and eveness (Brown and Weir 1983). A low SDI indicates an extremely unbalanced frequency classes for a character being studied and lack of genetic diversity. The results suggest that there is adequate allelic diversity for the seed quality traits of interest. However, to obtain more conclusive results, the trials may have to be repeated over a number of years and at several locations.

4.5 CONCLUSIONS

Variations exist among mini core accessions for seed colour, shape, seed diameter, TSW, protein and starch, but the level of phenotypic diversity is low. Mean concentrations of amylose, starch, and protein concentration, seed diameter and TSW were 27.6±1.2%, 51.2±2.2%, 26.2±1.3%, 5.7±0.5mm and 158±31.6g, respectively, in desi from across all regions. In kabuli, mean amylose, starch and protein concentration, and seed diameter, TSW were, respectively 27.0±1.1%, 47.6±3.0%, 22.5±1.6%, 6.8±0.5mm and 237.8±50.2g. SDI suggests that despite the narrow range of phenotypic diversity, the minicore collection has allelic diversity and evenness, which needs to be expressed to obtain the increased phenotypic diversity. In this study, some desi and kabuli accessions showed increases in some quality traits over the CDC released varieties and advanced breeding lines. This study suggests that, mini core collection should be

tested over three to four years at three or four locations with diverse growing conditions to obtain the adequate expression of all the allelic combinations. Seed composition traits are strongly influenced by environment (chapter 3), therefore a multi location and multi-year trials will be needed to identify genotypes of interest for use in chickpea improvement programs.

CHAPTER 5

CHICKPEA (*Cicer arietinum* L.) STARCH STRUCTURE AND ITS RELATIONSHIP TO STARCH HYDROLYTIC ACTIVITIES IN EXTRACTED STARCH AND MEAL.

5.1 ABSTRACT

An important indicator of starch quality is its rate of enzymatic digestion which is affected by starch structure: amylose to amylopectin ratio, amylopectin chain length distribution and granule size. The objective of this study was to investigate the effects of starch structure on rate of starch enzymatic hydrolysis of selected desi and kabuli chickpea mini core genotypes. Meal starch hydrolytic rate did not show any relationship with starch amylose concentration in both desi and kabuli. Starch concentration was slightly higher in desi than kabuli as desi seeds were dehulled. Amylose concentration did not significantly differ between the two market classes. More than 50% of analyzed starch granules were in the GS5 group, followed by GS20 (between 30-49%), GS40 (<10%) and GS60% (<10%) in both chickpea market classes. Desi and kabuli class starch showed a similar distribution with least proportion of C chains and increasing proportion of A, B2 and B1 chains, respectively. Both meal and extracted starch showed highest rate of hydrolysis at 20 min of incubation and the lowest rate of hydrolysis at 240 min of incubation across desi and kabuli. Seed meal showed a higher HI in both desi (12.4-36.8) and kabuli (38.6-50.3) compared with extracted starch in desi (22.4-24.7) and kabuli (24.9-47.2). Limited results on starch concentration, composition and structural characteristics in three desi and three kabuli genotypes based on cluster analysis suggest that desi and kabuli genotypes do not differ in their starch characteristics. Multivariate analysis of starch characteristics and extracted starch hydrolytic properties did not show any difference between desi and kabuli type genotypes. Observed changes in starch properties by interactions with seed meal constituents may have a major impact on chickpea seed utilization in food and feed applications.

5.2 INTRODUCTION

Legumes are an important component of human diet in majority of the population in developing countries. Starch is the major storage carbohydrate accounting for up to half of the chickpea seed dry weight. Upon consumption by humans, starch is hydrolysed in the digestive tract and is

a major source of energy for metabolic functions. A part of starch that is not digested in the small intestine, but is fermented by colonic microflora in the large intestine is called resistant starch (RS). Starch hydrolysis during the digestive process is a key factor controlling glycemic index (Zhang et al., 2008). Jenkins et al., (1981) proposed the concept of 'Glycemic Index (GI)' to characterize the blood glucose raising potential of carbohydrates in food. Pulses were identified as a low GI food nearly three decades ago (Jenkins et al., 1981; Thorne et al., 1983). Dietary fiber and RS have low GI and are the major contributors to beneficial effects of pulses in human health. In most foods, the RS proportion is low (typically 0 – 5% of starch in cereal foods), but pulses contain a relatively higher RS proportion (10-20% in some beans). In a recent comparative study of nine crops, pulses were found to be a better source of RS over cereals and a pseudocereal (Mikulikova and Kraic, 2006).

Starch composition and structure affects the rate of starch enzymatic digestion, therefore, it is an important factor to optimize starch quality for human health benefits (Gallant et al., 1992). Several plant studies have demonstrated the relation between starch composition and its rate of hydrolysis (Skrabanja et al., 1999; Vesterinen et al., 2002; Tester et al., 2004). Pea with high amylose starch showed a reduced hydrolytic index and a low glycemic index as compared to pea starch with normal amylose concentrations (Skrabanja et al., 1999). However, in rice, amylose concentration did not affect RS levels, as significant differences in RS were observed in mutants with similar amylose concentration. Interestingly, mutants high in RS had significantly increased proportions of short amylopectin chains (DP$\leq$ 12), and decreased numbers of intermediate (DP$\leq$ 13-36) and long chains (DP$\leq$ 37) (Shu et al. , 2009). In selected legume seed starches including chickpea, starch enzymatic hydrolysis was negatively correlated with starch granule diameter, and molecular weight of amylose and amylopectin (Sandhu and Lim, 2008). However there are no studies showing the effect of amylopectin starch on starch hydrolysis rates and/or and differences in amylopectin structure between desi and kabuli-type chickpea. The main objective of this study was to analyze selected desi and kabuli chickpea genotypes for starch concentration, granule composition and size, and amylopectin structure and see the effect of starch concentration and composition on seed meal and starch hydrolysis.

5.3 MATERIALS AND METHODS

5.3.1 Genotypes and growing conditions

Nine genotypes, each from desi and kabuli-type chickpea from diverse regions (Table 5.1) were selected based on amylose concentration for an initial screen of seed meal starch enzymatic hydrolytic activity. Subsequently, three desi (ICC12824, ICC9848 and CDC Cabri) and kabuli (ICC9862, ICC2277 and FLIP97-133C) were selected for detailed starch composition, structure analysis and and starch hydrolytic activity determinations.

The eighteen selected desi and kabuli accessions were grown in unreplicated trials at Davidson, Saskatchewan (51°25'N - 105°48'W), Kyle (50°49'N - 108°1'W) and Saskatoon (SPG) (52°09'N - 106°36'W), all in Saskatchewan, Canada in the summer of 2007. Seeding was done on May 22, 23 and 24 2007 at Kyle, Davidson and Saskatoon, respectively. Davidson, Kyle and Saskatoon sites belong to the Chernozemic Order with 1-17% organic carbon, C/N ratio of less than 17 and dominant in Ca (Pennock, 2006). Kyle is located within the brown soil zone, while Davidson and Saskatoon are in the Dark Brown Soil Zone of western Canada. The mean temperatures (May-August) in 2007 were 15.8, 15.4 and 16.6 ^{O}C for Davidson, Kyle and Saskatoon, respectively. However, total precipitation (May-August) was 198.0, 234.0 and 274.4 mm in 2007 for Davidson, Kyle and Saskatoon, respectively. Plot size was 1 m^2 with 30 cm distance between adjacent plots. Fifty seeds were sown in three short rows for each plot. Seeds were treated with fungicide Apron FL® before planting to protect from soil- borne pathogens. Crop maintenance in field followed the standard cultural practices for the area: hand weeding and controlling ascochyta blight (*Ascochyta rabiei*) with an application of Prothioconazole (Proline 480 SC) at 150 mL per acre.

5.3.2 Dehulling and grinding of chickpeas

Desi seeds were dehulled and ground whereas kabuli seeds were ground intact as described in section 3.3.3.

5.3.3 Isolation of starch granules

Dehulled seeds (10g) were steeped overnight in 50 mL plastic tubes containing 40 mL of 0.2% sodium hydrogen sulphate (0.2% $NaHSO_4$) at 40°C. After decanting the steeped solution,

Table 5.1: Description and origin of selected chickpea genotypes used to analyze seed meal hydrolytic activity.

Market Class	Genotype	Origin
Desi	ICC637	India
Desi	ICC1356	India
Desi	ICC2720	Iran
Desi	ICC7184	Turkey
Desi	ICC9848	Afghanistan
Desi	ICC12824	Ethiopia
Desi	ICC14077	Ethiopia
Desi	ICC14595	India
Desi	CDC Cabri	CDC, Canada
Kabuli	ICC2277	Iran
Kabuli	ICC5879	India
Kabuli	ICC7308	Peru
Kabuli	ICC8151	USA
Kabuli	ICC9862	Afghanistan
Kabuli	ICC13077	India
Kabuli	ICC13461	Iran
Kabuli	ICC15435	Morocco
Kabuli	FLIP97-133C	ICARDA, Syria

the grains were made into a slurry using pestle and mortar. The slurry was filtered through 70-mesh nylon cloth and washed thoroughly with nano-pure water. The filtrate was centrifuged at 2000 x g for 20 min, and the supernatant was carefully removed by aspiration. Pellet was washed three times with 95% (v/v) ethanol, centrifuged at 2000 x g, 20 min and supernatant was decanted each time. Subsequently, the pellet was washed three times with 40 mL of 0.2% (w/v) NaOH, and centrifuged at 2000 x g, 20 min and supernatant was removed each time. Pellet was washed once more with 40 mL Wash Buffer [55 mM Tris-Hcl, pH 6.8, SDS 2.3% (w/v); glycerol 10% (v/v); 9.5 mL wash buffer and 500 µl β-mercaptoethenol] solution, centrifuged at 2000 x g, for 20 min and the supernatant was carefully decanted. The pellet washed three times with nano-pure water, centrifuged at 2000 x g, for 20 min and supernatant decanted each time until starch was free of colour and the decanted supernatant was colourless. Finally, the pellet was washed with acetone, centrifuged at 2000 x g for 20 min and supernatant was carefully decanted. The starch was left overnight at room temperature to dry.

5.3.4 Amylose determination

Amylose concentration was determined after isoamylase mediated debranching of starch using a high performance size-exclusion chromatography (HP-SEC) method (Demeke et al., 1999) as described in section 3.3.6.

5.3.5 Starch granule size analysis

Starch granule size was determined using Malvern Mastersizer-2000 laser-diffraction analyzer (Malvern Instruments Ltd, Malvern, UK). Refractive indices of 1.31 for water and 1.52 for starch were used as standard. Starch (40 mg) was suspended in 1 ml water and dispersed in sample holder (1700 rpm) attached to the instrument and sonication was allowed during sample analysis. Laser obscuration was maintained between 12-14% during sample addition as recommended by the manufacturers. Each sample was analysed three times and average of three was recorded. Data was obtained as volume percentage of starch sample. Results were statistically analysed.

5.3.6 Determination of the α-1,4-glucan chain-length distribution by capillary electrophoresis

Amylopectin chain length distribution (DP 6-55) was analysed using fluorophore assisted capillary electrophoresis (FACE; Morell et al. 1998). Purified and defatted starch granules (20 mg) in a microfuge tube (2 ml) were suspended in 750 µL of distilled water and vortexed followed by addition of 50 µL of sodium hydroxide (2M). The suspension was boiled for 5 min, allowed to cool at room temperature and neutralized with 32 µL glacial acetic acid. Sodium acetate (1M, 100 µL) and distilled water (1 mL) was added to the gelatinized sample. Isoamylase (10 units) were added to de-branch the gelatinized starch at 37 °C for 2 h. After 2 h of incubation, the reaction mixture was placed in a boiling water bath to inactivate isoamylase activity and stop the de-branching reaction. The stopped reaction mixture was centrifuged at 3000 x g for 10 min and the supernatant was collected and deionized by filtration through an ion exchange resin (20-50 mesh, Bio-Rad, Mississauga, Ontario, Canada). An aliquot (50 µL) of the supernatant was dried in speed-vac (Thermo Savant, Holbrook, NY, USA) for 30 min. The dried sample was labeled with a fluorescent tag following manufacturer's protocol (ProteomLab™ Carbohydrate Labelling and Analysis Kit manual (Beckman Coulter, CA, USA). Briefly, the dried sample was mixed with 3.5 µL each of a fluorophore, 8-aminopyrene 1,2,6-trisulfonate (APTS) and sodium cyanoborohydride (1M solution in Tetrahydrofuran). The labeled samples were incubated overnight at 37 °C. The reaction was terminated next day with the addition of 43 µL of distilled water. The tubes were centrifuged at 3000 x g for 10 min, to remove insoluble material. To 5 µL of the labeled aliquot, 195 µL nano-pure water was added in the instrument sample vials and mixed before placing in the sample holder for capiilary electrophoresis. Maltose was used as internal standard. G-20 glucose ladder was used to determine degree of polymerization with respect to the retention time.

5.3.7 Resistant Starch Determination

5.3.7.1 Starch hydrolysis

Chickpea seed meal or purified starch was enzymatically hydrolyzed essentially as described (McCleary and Monaghan 2002), using a commercial Resistant Starch assay kit (K-RSTAR, Megazyme International Ireland Ltd., Wicklow, Ireland. Meal or starch (100 ± 5 mg) samples in screw-cap tubes (16x125 mm) were treated with 4.0 mL pancreatic α-amylase (10mg/mL)

containing amyloglucosidase (AMG) (3 U/ mL) in sodium maleate buffer (0.1M, pH 6), vortexed and incubated at 37 ^{o}C in a shaking water bath (~200 strokes min^{-1}; 1290-00 Yamato Scientific Co., Japan) for 20 min, 80 min, 2 hr and 4 hr. These timings allowed the estimation of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) after 2 and 4 hr, respectively (Englyst et al, 1992b). After the requisite incubation, 4.0 mL ethyl alcohol (95 % v/v) was added to the reaction mixture which was vortexed and centrifuged at 1300 x g for 10 min without caps on. Supernatant was decanted and pellet was suspended in 2 mL ethyl alcohol (50 %, v/v) with vigorous vortexing. An additional 6 mL ethyl alcohol (50 % v/v) was added, vortexed and centrifuged at 1300 x g for 10 min. Supernatant was decanted and the pellet was re-suspended in ethyl alcohol (50 %, v/v) and centrifugation step was repeated once more. After decanting the supernatant, the tubes were inverted on absorbent paper to drain all the liquid. The tubes were re-capped and, stored at 4 ^{o}C overnight.

5.3.7.2 Measurement of non-hydrolyzed starch

Each tube containing the non-hydrolyzed starch was suspended in 2 mL KOH (2M) and the contents were vigorously stirred with a magnetic stirrer (bars 5 x 15 mm) for 20 min. Starch slurry in each tube was mixed with 8 mL sodium acetate buffer (1.2 M, pH 3.8) followed by AMG (0.1 mL; 3300 U mL^{-1}) mixed by vortexing, and incubated for 30 min at 50 ^{o}C with intermittent vortexing. With the assumption that resistant starch of chickpea samples were >10%, reaction mixtures were quantitatively transferred to a volumetric flask (100 mL) and volume adjusted to 100 mL with water. An aliquot (1 mL) from each assay mixture was centrifuged (1400 x g) for 10 min. After centrifugation, an aliquot (0.1 mL) of the supernatant was treated with glucose oxidase/peroxidase (GOPOD) reagent (3.0 mL) and incubated for 20 min at 50 ^{o}C. Blank reaction contained sodium acetate buffer (0.1mL, 0.1M, pH 4.5) and GOPOD reagent (3.0 mL), whereas glucose standard reaction contained glucose (0.1 mL, 1 mg mL^{-1}, w/v) and GOPOD reagent (3. 0mL). Absorbance of sample solutions was read at 510 nm against a reagent blank.

5.3.7.3 Measurement of hydrolyzed starch

Pooled supernatants from each sample after the starch hydrolysis step was adjusted to 100 mL with water in a volumetric flask and mixed vigorously by shaking. Three aliquots (0.1 mL each)

were incubated with GOPOD reagent (3.0 mL) at 50 ^{o}C for 20 min. Absorbance was measured at 510 nm wavelength against a reagent blank as described before.

5.3.7.4 Calculations

Resistant Starch (g/100g sample):

= ΔE x F x 100/0.1 x 1/1000 x 100/W x 162/180= ΔE x F/W x 90

Non-Resistant (Solubilized) Starch (g/100g sample):

= ΔE x F x 100/0.1 x 1/1000 x 100/W x 162/180= ΔE x F/W x 90

Total Starch = Resistant Starch + Non-Resistant Starch

where:

ΔE = absorbance (reaction) read against reagent blank

F = conversion from absorbance to microgram (absorbance obtained for 100 µg of glucose in the GOPOD reaction is determined and F=100 (µg of glucose) divided by the GOPOD absorbance for this 100µg of glucose.

100/0.1 = volume correction (0.1 mL taken from 100mL)

1/1000 = conversion from microgram to milligrams

W = dry weight of sample analyzed

100/W = factor to present RS as a percentage of sample weight

162/180 = factor to convert from free glucose, as determined, to anhydro-glucose as it occurs in starch

5.3.7.5 Determination of starch hydrolysis index

The kinetics of starch hydrolysis was described by a non-linear model proposed by Goni et al. (1997). The first order equation was used to determine the area under the hydrolysis curve (AUC) as follows:

$$AUC = C_\infty (t_f - t_0) - (C_\infty/k)[1 - \exp{-k(t_f - t_0)}]$$

Where C_∞ is the concentration at equilibrium (t_{240}), t_f is the final time at 240 min, t_0 is the initial time at 0 min, and k is a kinetic constant. Hydrolysis index was estimated by dividing the area under the hydrolysis curve of each sample by area of a reference sample.

5.3.8 Statistical analysis

Data was analysed using proc GLM of statistical analysis system (SAS version 9, SAS Institute Inc., Cary, NC, USA). Multivariate similarity and correlation coefficient distance analyses were done using Minitab (version 16).

5.4 RESULTS AND DISCUSSIONS

5.4.1 Screening chickpea genotypes for starch hydrolytic activity

Seed meal from nine desi-type chickpea genotypes grown at three different locations was assayed for starch hydrolytic activity. Most of the genotypes exhibited similar starch hydrolytic activity, except CDC Cabri, which had considerably reduced starch hydrolytic activity (Figure 5.1). ICC9848 showed the highest and ICC12824 showed intermediate starch hydrolytic activity. Seed meal starch hydrolytic activity did not show any relationship to starch amylose concentration as 28.1, 26.2 and 25.8 % amylose was observed in ICC12824, CDC Cabri and ICC9848, respectively (Table 5.2).

Screening of seed meal from nine kabuli-type chickpea genotypes also did not show much variation in starch hydrolytic activity. Among the observed variation, FLIP97-133C showed the highest starch hydrolytic activity, followed by ICC2277 and ICC9862, which had the lowest starch hydrolytic activity (Figure 5.2). As in desi-type chickpea, in kabuli types also starch hydrolytic activity was not related to seed starch amylose concentration (Table 5.2). These observations that starch hydrolytic acivity is not related to starch amylose concentration are similar to those in rice (Shu et al 2009). Therefore, within available variation in starch hydrolytic activities, three desi genotypes (CDC Cabri, ICC12824 and ICC9848) and three kabuli genotypes (FLIP97-133C, ICC2277 and ICC9862) were selected for analysis of starch granule size and composition and amylopectin structure determination and their influence on starch hydrolytic activities in seed meal and extracted starch.

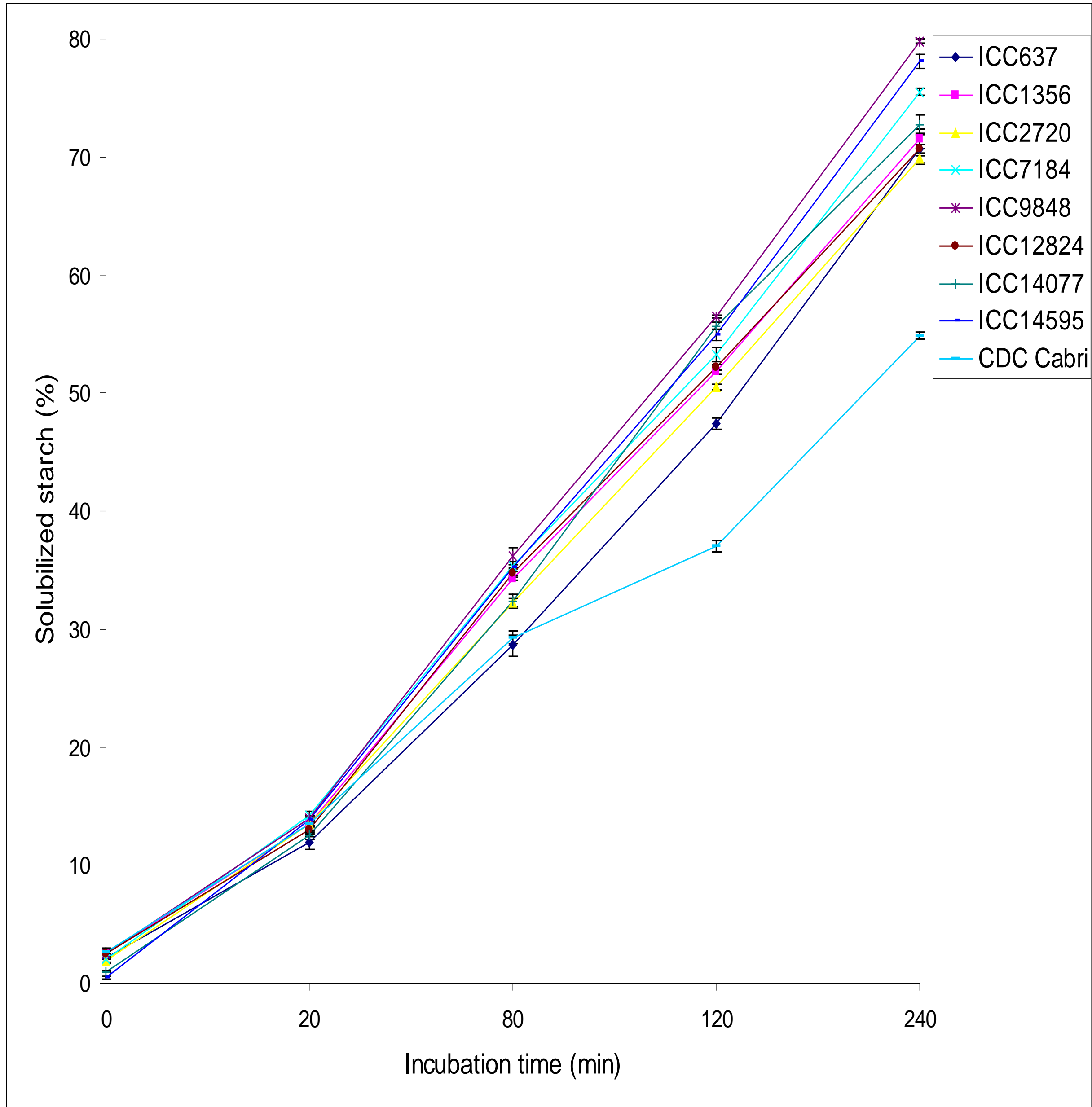

Figure 5.1 Rate of meal starch hydrolysis of selected desi chickpea grown across three environments in Western Canada.

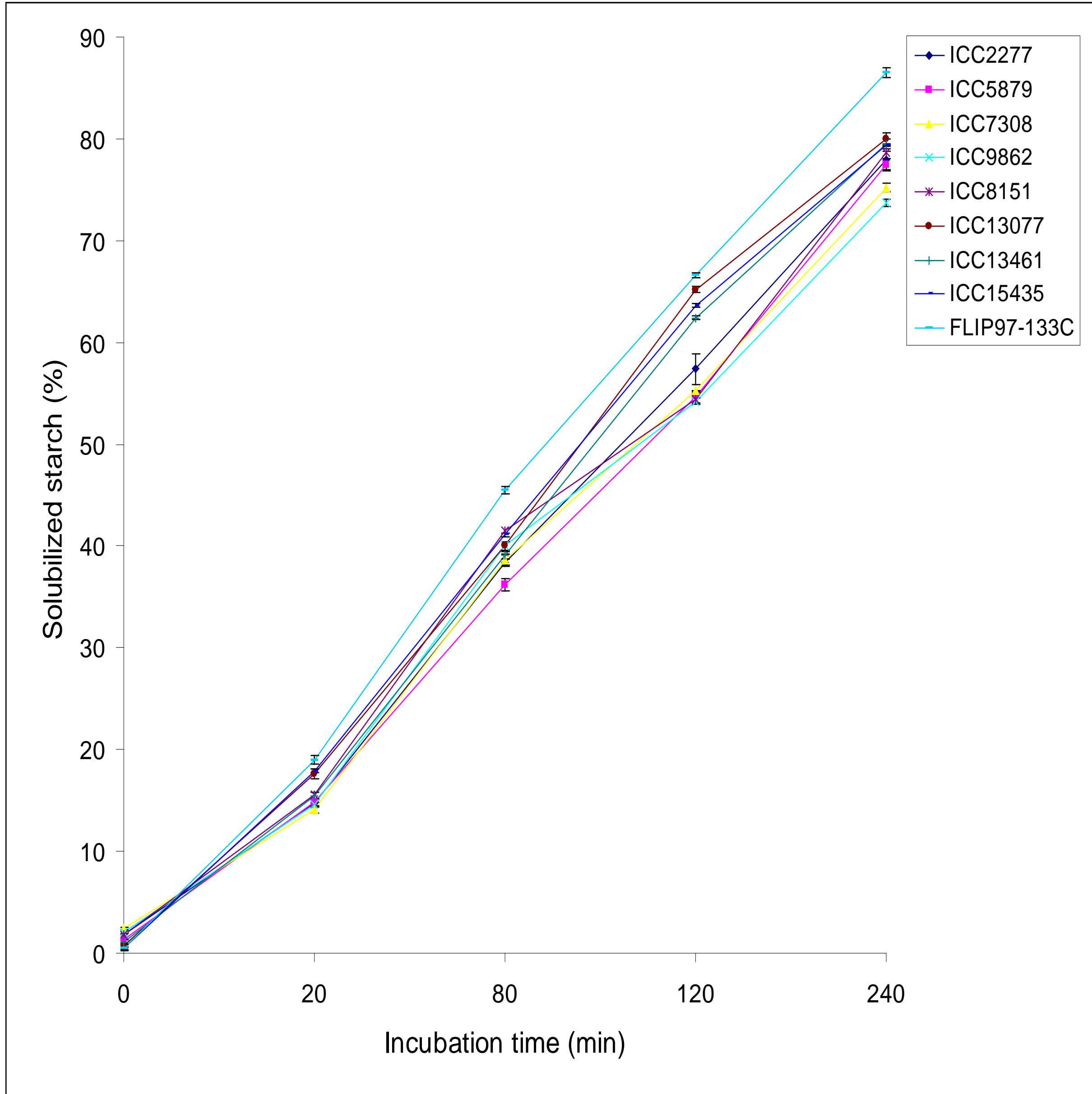

Figure 5.2 Rate of meal starch hydrolysis of selected kabuli chickpea grown across three environments in Western Canada.

Table 5.2. Mean (±sd) and ranges of starch and amylose concentration in selected chickpea genotypes.

Market Class	Genotype	Total starch (%)	Amylose (%)
Desi	CDC Cabri	51.6±2.9 46.8-57.8	26.2±1.3 24.5-28.2
Desi	ICC9848	52.0±2.9 49.7-54.1	25.8±1.3 24.1-27.2
Desi	ICC12824	48.2±2.9 46.0-49.5	28.1±1.3 24.8-30.1
Kabuli	ICC2277	49.2±3.6 47.3-52.1	27.4±1.3 25.8-28.2
Kabuli	ICC9862	48.0±3.6 47.0-49.4	25.2±1.3 24.1-26.2
Kabuli	FLIP97-133C	49.0±3.6 44.8-53.0	26.6±1.3 24.8-28.3

Table 5.3. Amylopectin chain length distribution in amylopectin fraction of selected chickpea genotypes.

Type	Genotype	A[%] (6-12)	B1[%](13-25)	B2[%](26-35)	C[%](36-55)
Desi	CDC Cabri	7.61b	69.49a	19.23	3.67
Desi	ICC9848	23.31a	56.99b	15.41	4.30
Desi	ICC12824	8.62b	69.93a	17.38	4.07
Kabuli	ICC2277	10.88b	68.38a	16.52	4.22
Kabuli	ICC9862	7.97b	69.50a	18.62	3.91
Kabuli	FLIP97-133C	20.39a	57.09b	17.11	5.41
Lsd		4.42	5.60	2.69	1.42
Sem		2.57	3.26	1.56	0.82

Variation in different chickpea lines with respect to percent proportions of four different chains A (dp 6-12), B1 (dp 13-25), B2 (dp 26-35) and C (dp 36-55) was analyzed by ANOVA. Variation within each of the first two columns was evaluated, and numbers followed by same letter are not statistically different ($P<0.05$, Tukey's HSD). Variations in percent proportions of B2 and C chain were not significant.
Group 1----FLIP97-133C (Kabuli), ICC9848 (Desi).
Group 2----ICC9862 (Kabuli), ICC2277 (Kabuli), CDC Cabri (Desi), ICC12824 (Desi).

5.4.2 Total starch and amylose concentration

Seeds from the three desi (CDC Cabri, ICC9848 and ICC12848) and kabuli (ICC2277, ICC9862 and FLIP97-133C) genotypes were analysed for total starch and amylose concentration. The results are based on three technical replicates from two independent biological replicates. The total starch concentration among all the genotypes varied from 44 to 57% (Table 5.2). The desi genotypes showed slightly higher starch concentration with average concentration varying from 48 to 52%. The kabuli genotypes showed an average starch concentration between 48 to 49%. The desi chickpea genotypes were dehulled before grinding therefore the starch concentration is slightly higher than kabuli genotypes. The amylose concentration varied from 24 to 30% among all the chickpea genotypes. However, the average amylose concentration did not significantly differ between the two market classes or individual genotypes (Table 5.2).

5.4.3 Starch granule size

The chickpea starch granules ranged in size from 5 to 80 µm, with no difference between the desi and kabuli genotypes. Since granule size categorization is not very well established in literature, these were then grouped into four classes 5-20µm (GS5), 20-40µm (GS20), 40-60µm (GS40) and 60-80µm (GS60) (Figures 5.3 and 5.4). In both desi and kabuli types more than 50 % starch granules were in the GS5 group, followed by the GS20 group. In these two groups the genotypes did not significantly ($P>0.05$) differ from each other in the GS20 for desi and GS5 for kabuli. The other two groups, GS40 and GS60 were less than 10% each in all the genotypes. In the desi genotypes ICC12824 had the smallest percentage of both the 40-60 µm and 60-80 µm starch granules (Figure 5.3). In the kabuli genotypes ICC9862 had the lowest number of starch granules with diameter larger than 40 µm whereas FLIP97-133C had the lowest number of starch granules with diameter larger than 60 µm. However, using Laser light scattering particle size analyzer (1064 LD, CILAS, France), Sandhu and Lim (2008) reported chickpea granule size distribution to be in the range 0-50µm. The differences between our results and that of Sandhu and Lim (2008) may be explained by differences in genetic backgrounds, starch isolation procedures as well as instrument used to determine starch granule size.

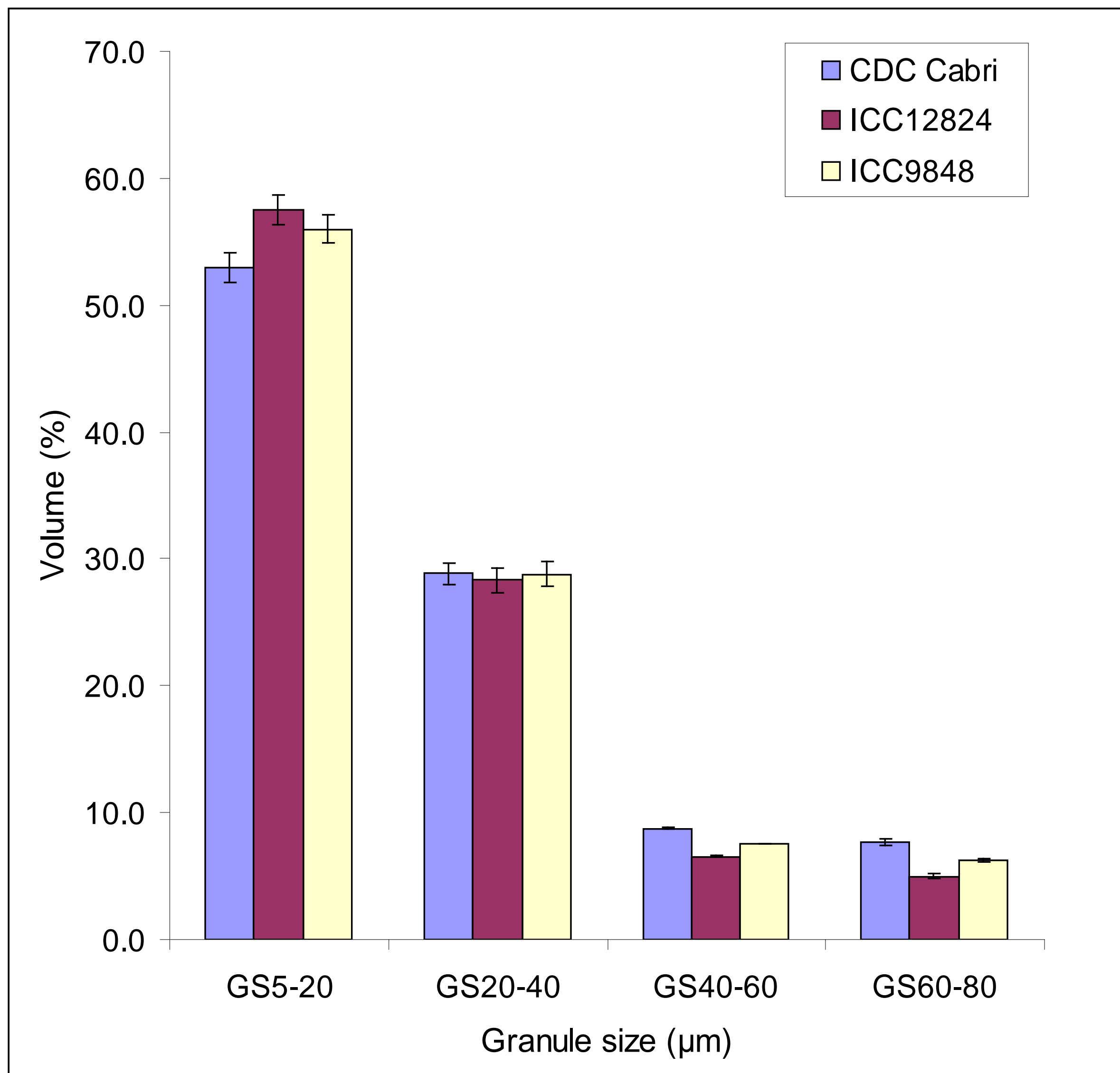

Figure 5.3: Starch granule size distribution in selected desi chickpea genotypes.

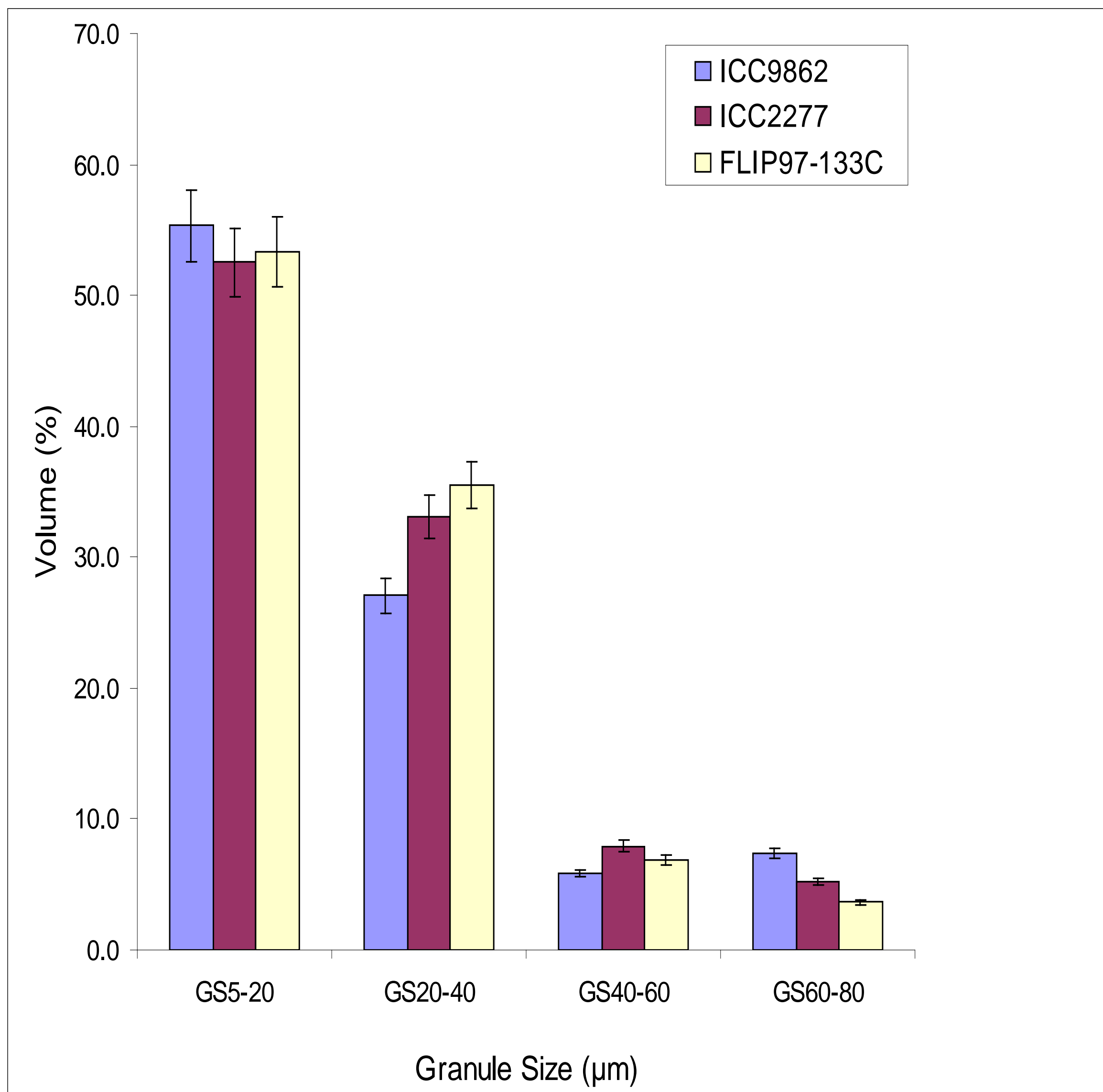

Figure 5.4: Starch granule size distribution in selected kabuli chickpea genotypes.

5.4.4 Amylopectin chain length

The amylopectin chain lengths determined by FACE analysis were grouped into A, B and C chains (Hanashiro et al 1996). The A, B and C chains included polymers with DP 6-12, 13-35 and 36 to 55 or higher, respectively (Table 5.3). The B chains were further sub-divided into B1 and B2 with DP 13-25 and 26-35, respectively. In general both desi and kabuli type starch showed similar distribution, with least proportion of C chains, with increasing proportions of A, B2 and B1 chains, respectively. However, desi genotype ICC9848 and kabuli genotype FLIP97-133C showed higher proportion of A than B2 chains (Table 5.3). Kabuli genotype FLIP97-133C was also unique because it had the highest proportion (5.41%) of C chains among all the genotypes studied. Desi genotype ICC9848 had the highest proportion (4.3%) of C chains among the desi genotypes. ANOVA of amylopectin structure revealed two interesting groupings ($P<0.05$, Table 5.3) of chickpea types: FLIP97-133C (kabuli), ICC9848 (desi) in group 1 and ICC9862 (kabuli), ICC2277 (kabuli), CDC Cabri (desi) in group 2. Group 2 contains ICC2277 (kabuli) and CDC Cabri (desi) both having comparatively lower rates of hydrolysis and potential prebiotic effects.

5.4.5 Rate of starch hydrolysis

A RS control supplied with the kit, showed maximum rate of starch hydrolysis after 20 min of incubation, with a considerably lower but similar rate of hydrolysis at subsequent time intervals of 80, 120 and 240 min, respectively. In desi chickpea meal or extracted starch, also highest rate of hydrolysis was observed at the initial 20 min of incubation (Tables 5.4 and 5.5). In two accessions ICC12824 and ICC9848 as compared to extracted starch, seed meal showed a higher rate of hydrolysis at 20, 80 and 120 min of incubation. At 240 min of incubation, rate of starch hydrolysis did not differ between meal and extracted starch in ICC9848, but compared to extracted starch, seed meal from ICC12824 showed slightly lower rate of hydrolysis (Tables 5.4 and 5.5). CDC Cabri, a cultivar adapted to Saskatchewan growing conditions behaved differently. At 20 min of incubation, as compared to purified starch the meal hydrolyzed starch at a lower rate which was also the lowest among the three genotypes studied (Tables 5.4 and 5.5). Whereas, CDC Cabri extracted starch at 20 min of incubation showed higher rate of hydrolysis and behaved similar to the two other genotypes (Table 5.5).

Table 5.4. Rate of hydrolysis (mg glucose produced per hour) of desi chickpea meal at 20, 80, 120, and 240 minutes of incubation with pancreatic α-amylase and amyloglucosidase.

	Time of incubation (min)			
Genotype	20	80	120	240
ICC12824	31.5a	21.8a	26.2a	9.2b
ICC9848	34.6a	22.2a	30.4a	11.6a
CDC Cabri	14.8b	6.9b	5.0b	3.6c
RS control	11.4c	3.3c	3.5c	3.1c
Mean	23.1	13.5	16.2	6.9
s.e.m	5.8	4.9	7.0	2.1

s.e.m- standard error of the means.
Means followed by the same letter within a column are not significantly different ($P<0.05$).

Table 5.5. Rate of hydrolysis (mg glucose produced per hour) of desi chickpea extracted starch at 20, 80, 120, and 240 minutes of incubation with pancreatic α-amylase and amyloglucosidase.

	Time of incubation (min)			
Genotype	20	80	120	240
ICC12824	21.8a	10.4a	18.0a	13.3a
ICC9848	21.7a	8.3b	16.6a	10.2b
CDC Cabri	23.5a	9.6ab	12.4b	10.9b
RS control	11.4b	3.2c	3.5c	3.1c
Mean	19.6	7.9	12.6	9.4
s.e.m	2.8	1.6	3.3	2.2

s.e.m- standard error of the means.
Means followed by the same letter within a column are not significantly different ($P<0.05$).

Table 5.6. Rate of hydrolysis (mg glucose solubilized per hour) of kabuli chickpea meal at 20, 80, 120, and 240 min of incubation with pancreatic α-amylase and amyloglucosidase.

Genotype	Time of incubation (min)			
	20	80	120	240
ICC9862	37.4b	25.5a	21.3b	9.7a
ICC2277	38.7b	23.8a	28.4a	10.3a
FLIP97-133C	55.6a	26.4a	31.7a	10.0a
RS control	11.4c	3.3b	3.5c	3.1b
Mean	35.8	19.7	21.2	8.3
s.e.m	9.1	5.5	6.3	1.7

s.e.m- standard error of the means.
Means followed by the same letter within a column are not significantly different ($P<0.05$).

Table 5.7. Rate of hydrolysis (mg glucose solubilized per hour) of kabuli chickpea extracted starch at 20, 80, 120, and 240 min of incubation with pancreatic α-amylase and amyloglucosidase.

Genotype	Time of incubation (min)			
	20	80	120	240
ICC9862	37.0b	12.0b	22.5a	10.0a
ICC2277	25.3c	9.0c	16.7b	9.2a
FLIP97-133C	56.3a	16.5a	13.2b	9.3a
RS control	11.4d	3.3d	3.5c	3.1b
Mean	32.5	10.2	13.9	7.9
s.e.m	9.5	2.8	4.0	1.6

s.e.m- standard error of the means.
Means followed by the same letter within a column are not significantly different ($P<0.05$).

Kabuli genotypes both meal and extracted starch showed the highest rate of hydrolysis at 20 min of incubation. FLIP97-133C showed the highest rate of starch hydrolysis 55.6 mg glucose h^{-1} solublized in meal, which was comparable to the amount of glucose solubilized in extracted starch (Tables 5.6 and 5.7). In ICC2277, the amount of glucose solubilized at 20 min was higher in meal as compared to extracted starch (Tables 5.6 and 5.7). In both meal and extracted starch, lowest rate of starch hydrolysis were observed at 240 min of incubation, except in ICC2277 extracted starch where the lowest rate of starch hydrolysis was observed at 80 min and with similar values at 240 min of incubation (Tables 5.6 and 5.7).

5.4.6 Starch digestibility studies and hydrolysis index (*HI*)

Hydrolysis of meal and extracted starch was used to calculate the proportions of rapidly digestible starch (RDS), slow digestible starch (SDS), resistant starch (RS) and hydrolytic index (HI) for the kabuli and desi types of un-cooked chickpea. In desi genotypes, the RS and SDS in desi seed meal ranged from 21.8-30.5% and 51.4-57.2%, respectively (Table 5.8). RS and SDS in extracted starch ranged between 40.0-43.8% and 41.6-44.9%, respectively (Table 5.9). No significant difference was detected among ICC 12824, ICC 9848 and CDC Cabri for both meal starch and extracted starch RS, RDS and SDS (Tables 5.8 and 5.9). The desi chickpea seed meal showed a broader HI ranging from 12.4-36.8 compared with extracted starch which had an HI of 22.4-24.7. CDC Cabri showed a lower HI both in meal and extracted starch. However, the HI was considerably lower in meal (12.4) as compared to extracted starch (22.4) (Tables 5.8 and 5.9). In the other two genotypes, ICC12824 and ICC9848, as compared to extracted starch the meal had a higher HI (Tables 5.8 and 5.9).

In kabuli chickpea genotypes, seed meal had lower concentrations of RS ranging from 22.4 to 25.6, but the SDS highest concentration did not differ between seed meal and extracted starch (Table 5.10). RS and SDS ranged from 30.0-38.9% and 40.5-53.5%, respectively in extracted starch (Table 5.11). However, ICC2277 and FLIP97-133C did show about 10% higher SDS in meal compared to extracted starch (Tables 5.10 and 5.11). Both in meal and extracted starch FLIP97-133C had higher concentration of RDS as compared to the two other kabuli genotypes. FLIP97-133C also had highest HI in seed meal and extracted starch, and the HI did not differ in meal of the ICC9862 and ICC2277. However, extracted starch from ICC2277

Table 5.8: Digestibility of starch and starch fractions from meal of desi chickpea genotypes.

Genotype	RS (%)	Digested starch (%)		HI
		RDS	SDS	
ICC12824	30.5b	18.1a	51.4a	34.5b
ICC9848	21.8b	21.0a	57.2a	36.8a
CDC Cabri	26.1b	18.1a	55.8a	12.4c
RS control	84.5a	5.5b	10.0b	9.6d

RS, resistant starch after 4 hrs of hydrolysis; RDS, rapidly digestible starch; SDS, slowly digestible starch; HI, hydrolysis index.
Means followed by the same letter within a column are not significantly different (P<0.05).

Table 5.9: Digestibility of starch and starch fractions from extracted starch of different desi chickpea genotypes.

Genotype	RS (%)	Digested starch (%)		HI
		RDS	SDS	
ICC12824	43.8b	12.5a	43.7a	24.7a
ICC9848	40.7b	17.7a	41.6a	23.7b
CDC Cabri	40.0b	15.1a	44.9a	22.4c
RS control	85.0a	5.0b	10.5b	9.6d

RS, resistant starch after 4 hrs of hydrolysis; RDS, rapidly digestible starch; SDS, slowly digestible starch; HI, hydrolysis index.
Means followed by the same letter within a column are not significantly different (P<0.05).

Table 5.10: Digestibility of starch and starch fractions from meal of different kabuli mini core chickpea genotypes.

Genotype	RS (%)	Digested starch (%)		HI
		RDS	SDS	
ICC 9862	25.6b	20.1b	54.3a	38.6b
ICC 2277	25.2b	18.5b	56.3a	39.6b
FLIP97-133C	22.4b	25.8a	51.8a	50.3a
RS control	84.5a	5.5c	10.0b	9.6c

RS, resistant starch after 4 hrs of hydrolysis; RDS, rapidly digestible starch; SDS, slowly digestible starch; HI, hydrolysis index.
Means followed by the same letter within a column are not significantly different ($P<0.05$).

Table 5.11: Digestibility of starch and starch fractions from extracted starch of different kabuli chickpea genotypes.

Genotype	RS (%)	Digested starch (%)		HI
		RDS	SDS	
ICC 9862	30.0b	16.5b	53.5a	34.9b
ICC 2277	38.9b	14.6b	46.5a	24.9c
FLIP97-133C	35.5b	24.0a	40.5a	47.2a
RS control	85.0a	5.0c	10.0b	9.6d

RS, resistant starch after 4 hrs of hydrolysis; RDS, rapidly digestible starch; SDS, slowly digestible starch; HI, hydrolysis index.
Means followed by the same letter within a column are not significantly different ($P<0.05$).

showed the lowest HI (24.9) followed by ICC9862 (34.9) and a maximum HI (47.2) was observed in FLIP97-133C (Table 5.11).

The higher HI in meal starch could be attributed to inherent α-amylase in the chickpea meal. CDC Cabri had significantly lower HI in both meal starch and pure starch ($P<0.05$) making it the best desi chickpea as prebiotic. This is imperative as HI is highly significantly correlated with glycemic index (GI) in chickpeas (Sandhu and Lim, 2008). Moreover with the exception of CDC Cabri which had HI of 12.4, HI values observed in these chickpea market classes for meal starch were higher than the 18 reported for chickpea genotypes from India (Sandhu and Lim 2008).

5.4.7 Desi and kabuli starch characteristics

Starch concentration, composition and structural characteristics in the three each of desi and kabuli genotypes (Table 5.12) showed two distinct clusters with 36.8% similarity (Figure 5.5). Starch concentration, which formed a cluster was related to starch granules (20 – 40 µm), but was very closely related (≥ 90%) to starch granules (40 – 60 µm). Starch concentration was also related to ratio of A/C amylopectin chains. The second cluster at ~58% similarity could be split into two clusters, one in which amylose concentration was related to the ratio of B1/B2 chains and very small starch granules (5- 20 µm). The second cluster in this group, included very large size starch granules (60 – 80 µm) which were closely related to fine amylopectin structure such as the ratio of B1/C, B2/C and B/A chains. The starch characteristics showed close to 95% similarity between all the six genotypes studied (Figure 5.6). FLIP97-133C (a kabuli genotype) was the most distinct among all the six, and both the kabuli and desi genotypes were interspsersed between the clusters.

A similar analysis for amylopectin structure revealed, that the ratio between A/C chains was most distinct in a cluster at 14.73% similarity (Figure 5.7). The second cluster included A and B chains and the ratio between these chains. The amylopectin structure did not differ among the six genotypes much and showed two groups with 96% similarity (Figure 5.8). Cluster I genotypes were about 99% similar consisting of CDC Cabri (desi), ICC9862 (kabuli), ICC12824 (desi) and ICC2277 (kabuli). CDC Cabri and ICC9862 were 100% similar for their amylopectin structures. Cluster II consisted of ICC9848 (desi) and FLIP97-133C (kabuli) which were more than 99% similar in their amylopectin structure mostly due to the closeness in their A and B1

chain length values. The limited results based on three genotypes of each class suggest that the desi and kabuli genotypes do not differ in their starch characteristics.

5.4.8 Relationship between starch hydrolytic activities and starch characteristics in chickpea meal and extracted starch

To study the relationship between starch characteristics and starch hydrolytic properties cluster analysis revealed two major groups with about 33.6% similarity (Figure 5.9). Forty nine percent (49%) similarity existed among components of cluster I (Figure 5.9). Two sub-clusters occurred under cluster I. In the first sub-cluster I, RDS was strongly associated with or influenced by HI, and starch granules between (20 – 40 µm). In the second sub-cluster I, starch was significantly affected by SGS40 and the ratio A/C chains in amylopectin. In the first sub-cluster II, Slowly digestible starch (SDS) was strongly influenced by starch granules (60 – 80 µm), amylopectin B1/C, B/C, B2/C and B/A. Resistant starch (RS) was strongly associated with amylose, B1/B2 and starch granules (5- 20 µm).

Seed meal, which contains starch, other non-starch polysaccharides, proteins and lipids when analyzed, also showed two major clusters with close to 35% similarity (Figure 5.10). The Cluster I at 51.3% similarity was split in two sub clusters. In the first subcluster, RDS was strongly influenced by B1/B2 amylopectin chain length ratios, amylose, hydrolysis index (HI) and very small starch granules (5 - 20 µm). In the second sub cluster RS was strongly influenced by large starch granules (60 – 80 µm), amylopectin B1/C, B/C, B2/C and B/A. Cluster II included SDS which was associated with amylopectin A/C, starch concentration and starch granules (40 – 60 µm). Cluster III also consisted of SGS20 which had low influence and similarity (56.7%) on SDS.

Table 5.12 Summary of starch hydrolytic activity in meal / extracted starch and amylopectin structure in selected chickpea genotypes used for analyses presented in Figures 5.5 to 5.11.

Genotype	RDS	SDS	RS	HI	Amylopectin chain ratios					
					B1/C	B2/C	B/C	B1/B2	B/A	A/C
CDC Cabri	18.1/ 15.1	55.8/ 44.9	26.1/ 40	12.4/ 22.4	18.93	5.23	24.17	3.61	11.61	2.07
ICC9848	21.0/ 17.7	57.2/ 41.6	21.8/ 40.7	36.8/ 23.7	13.25	3.6	16.83	3.69	3.11	5.42
ICC12848	18.1/ 12.5	51.4/ 43.7	30.5/ 43.8	34.5/ 24.7	17.18	4.27	21.40	4.02	10.12	2.11
ICC2277	18.5/ 14.6	56.3/ 46.5	25.2/ 38.9	39.6/ 24.9	16.20	3.91	20.11	4.13	7.80	2.57
ICC9862	20.1/ 16.5	54.3/ 53.5	25.6/ 30.0	38.6/ 34.9	17.77	4.76	22.54	3.73	11.05	2.03
FLIP97-133C	25.8/ 24.0	51.8/ 40.5	22.4/ 35.5	50.3/ 47.2	10.55	3.16	13.71	3.33	3.63	3.76

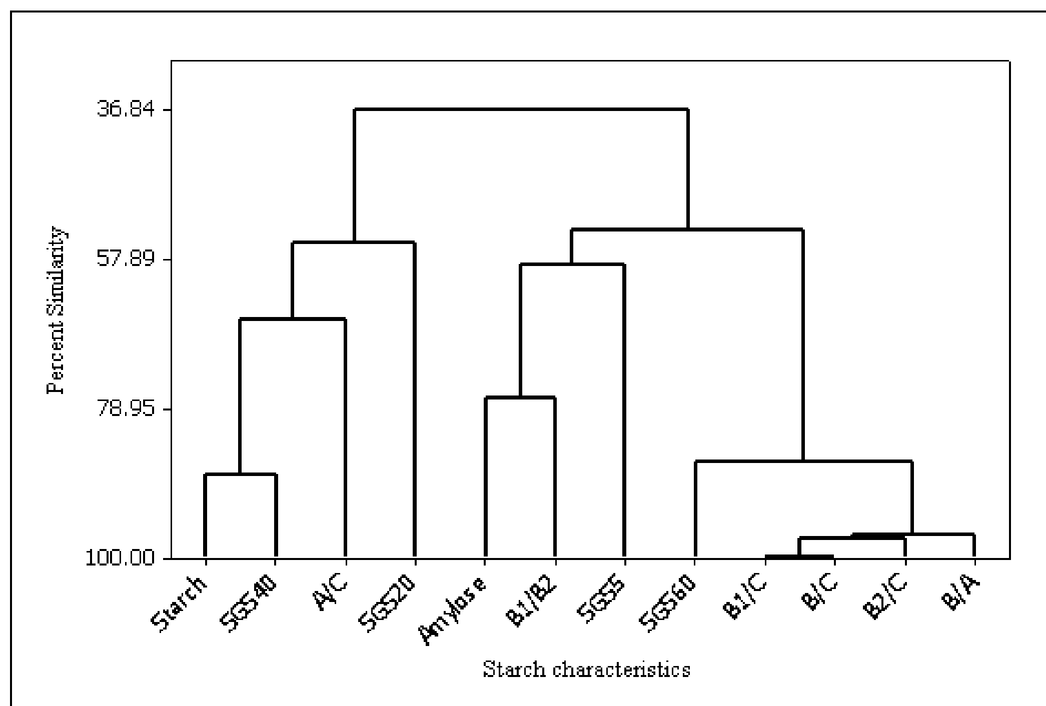

Figure 5.5 Average linkage and correlation coefficient distance in characteristics of starch extracted from selected chickpea genotypes.

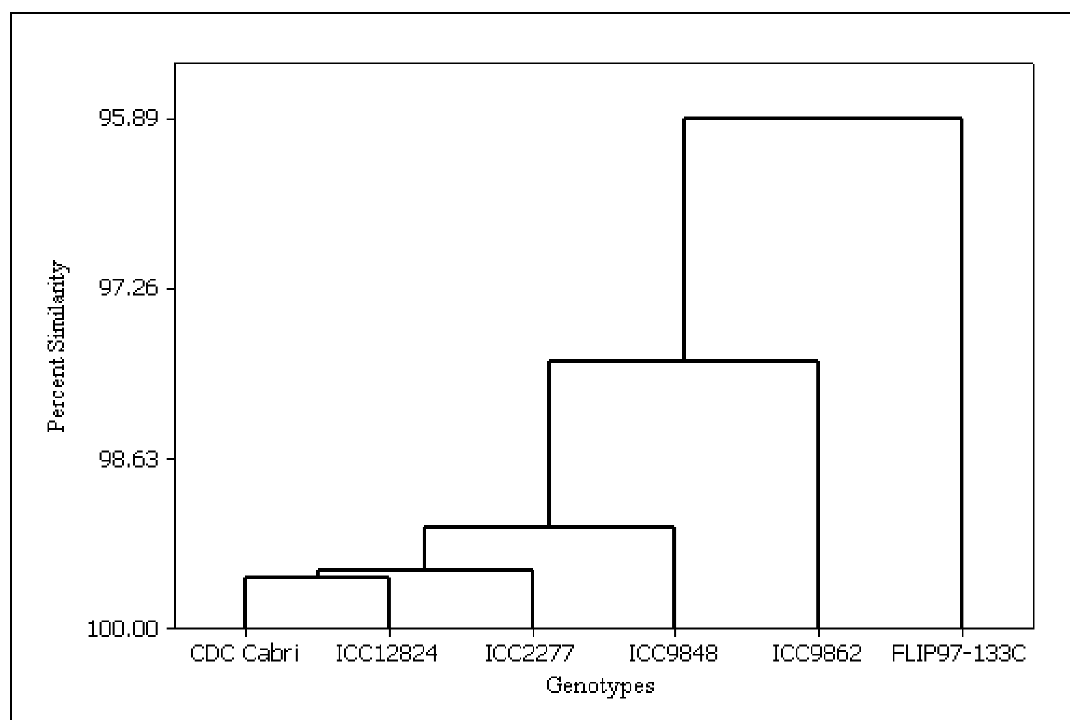

Figure 5.6 Average linkage and correlation coefficient distance among chickpea genotypes based on extracted starch characteristics

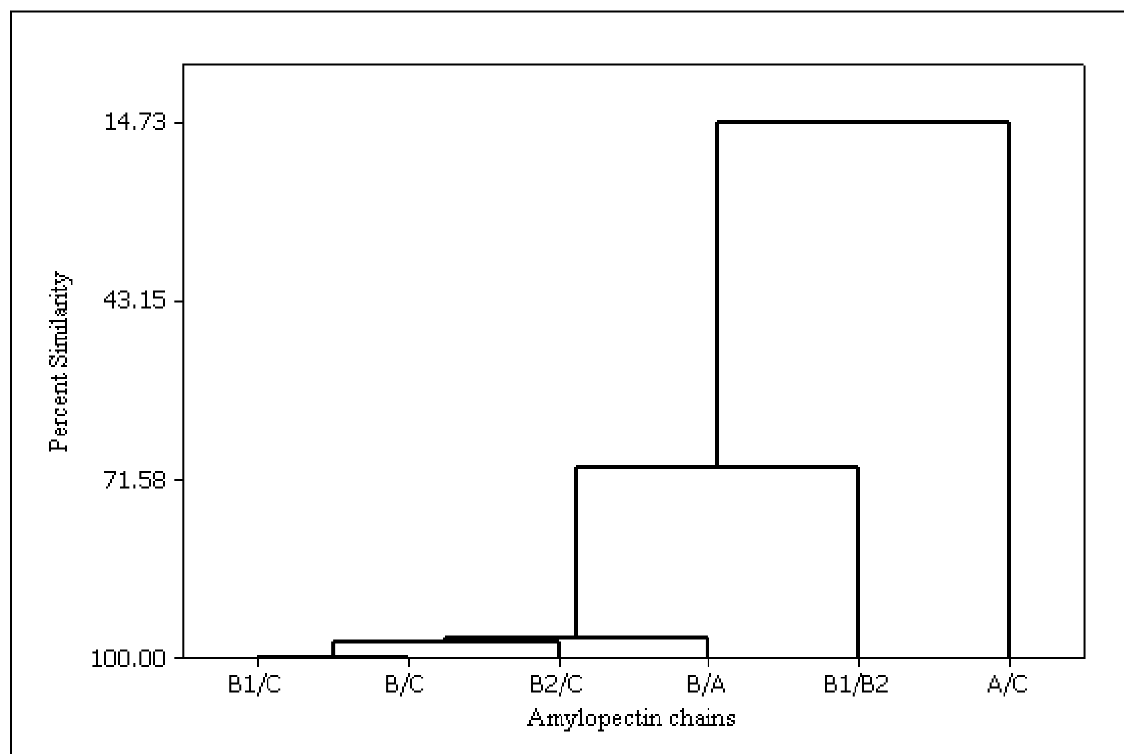

Figure 5.7 Average linkage and correlation coefficient distance in amylopectin chains based on extracted starch from selected chickpea genotypes

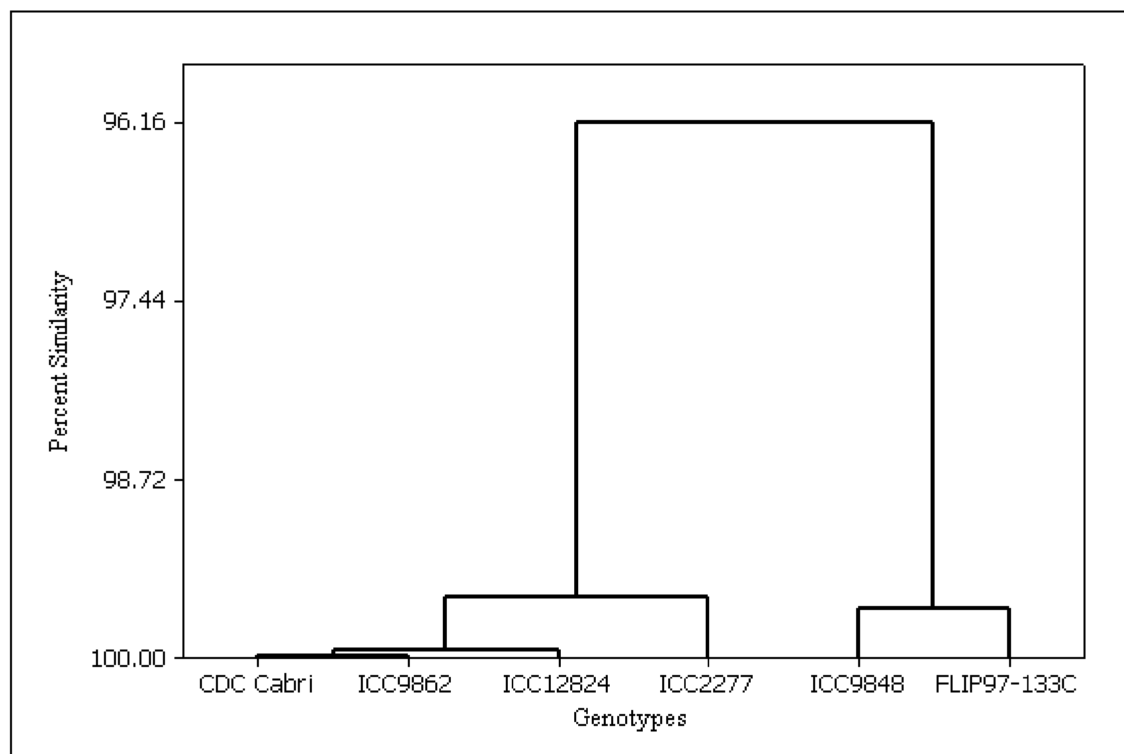

Figure 5.8 Average linkage and correlation coefficient distance among chickpea genotypes based on amylopectin structure of extracted starch.

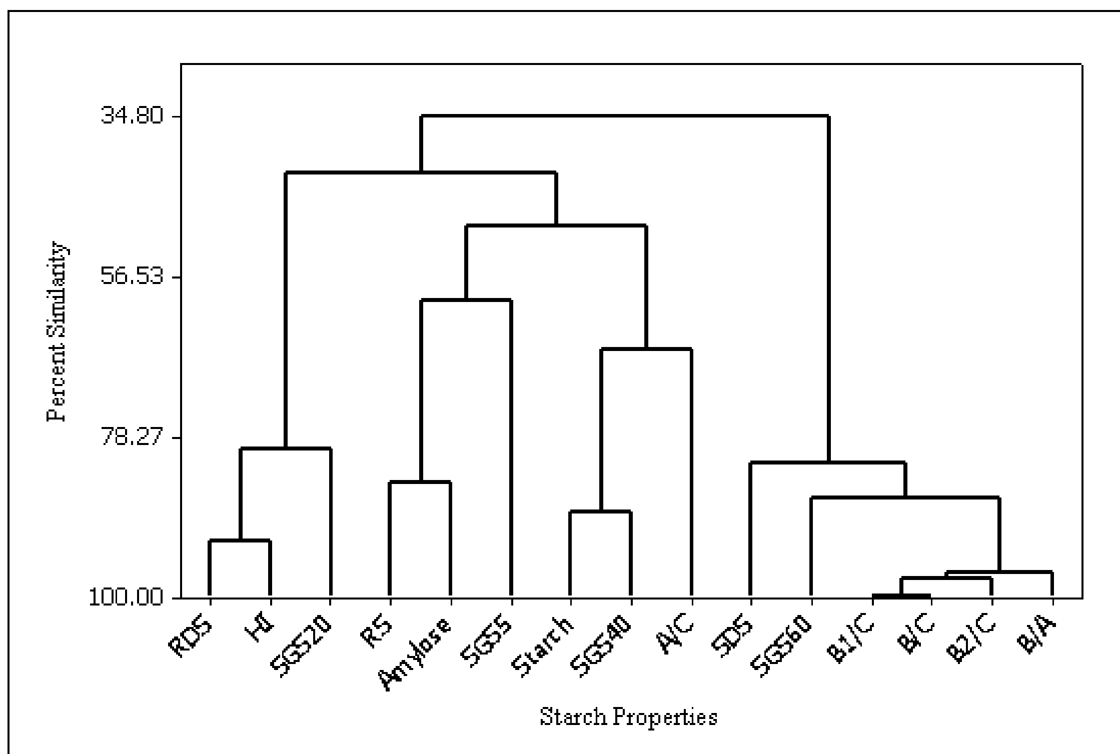

Figure 5.9 Average linkage and correlation coefficient distance in starch hydrolytic activities and starch characteristics based on extracted starch from selected chickpea genotypes.

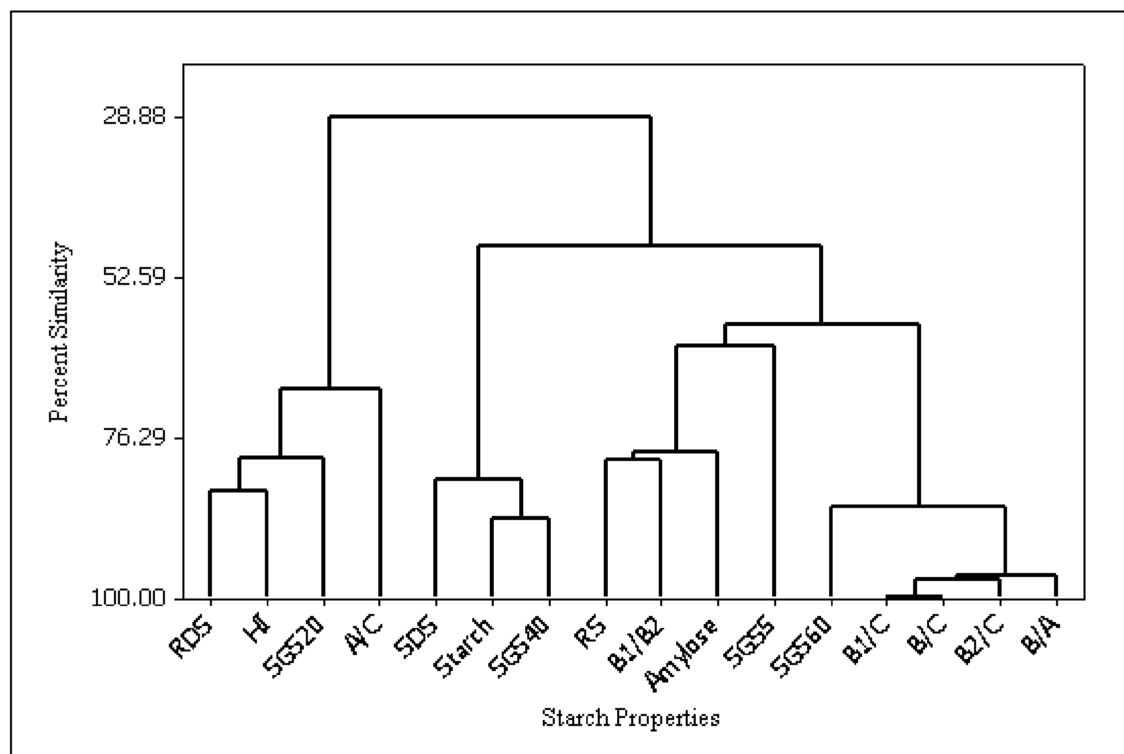

Figure 5.10 Average linkage and correlation coefficient distance in starch hydrolytic activities and starch characteristics based on meal prepared from selected chickpea genotypes.

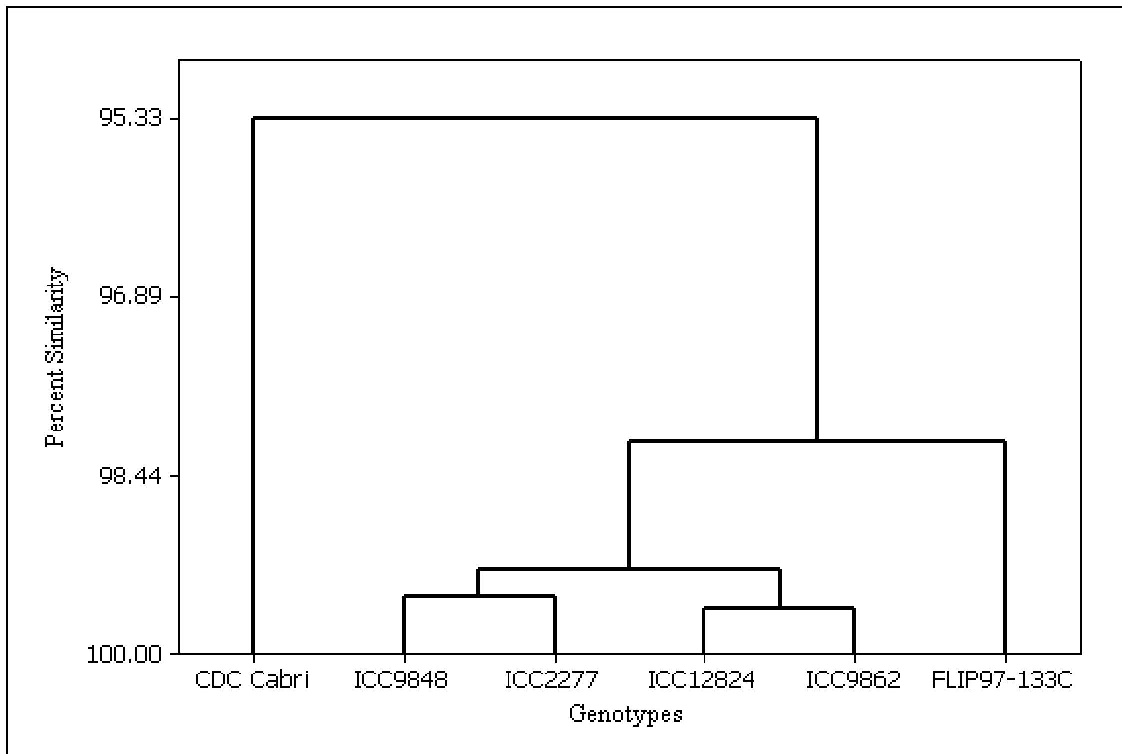

Figure 5.11 Average linkage and correlation coefficient distance among chickpea genotypes based on meal starch hydrolytic activities and starch characteristics.

The multivariate analyses of starch characteristics and extracted starch hydrolytic properties did not show any difference between the desi and kabuli type genotypes. Physical characteristics of extracted starch showed some interesting associations between starch concentration and starch granules (40 – 60 µm), ratio between A and C amylopectin chains and small starch granules (20 – 40 µm). Similarly, amylose was associated with the ratio of B1/B2 amylopectin chains and very small size starch granules (5 – 20 µm). The very large size starch granules were associated with the ratio of B to C amylopectin chains. These are interesting observations with a limited number of genotypes. To further confirm these findings, the study has to be expanded with diverse genotypes to draw any firm conclusions.

The starch hydrolytic properties revealed that RDS was closely associated with HI, and RS with amylose concentration. A strong positive correlation between HI and RDS in kabuli corroborates the findings of Sandhu and Lim (2008) for chickpeas. SDS, the most desirable form of dietary starch which is completely but more slowly digested in the small intestine and attenuates postprandial plasma glucose and insulin levels (Jenkins et al. 1981), is shown to be associated with amylopectin structure (Figure 5.9). This is an interesting observation because both long and short chains of amylopectin have been associated with maize SDS (Zhang et al 2008).

Meal is a complex mixture of protein, non-starch carbohydrates, lipids and other minor components. The seed constituents alter the starch hydrolytic properties. The RDS was associated with amylose concentration, HI, very small starch granules and the ratio of B1/B2 amylopectin chains. RS was found to be associated with large size starch granules and amylopectin B and C chains. SDS was found to be associated with starch concentration, ratio of short to long amylopectin chains and medium size starch granules. The observed change in starch properties by interaction with seed meal constituents will have a major impact on chickpea seed utilization in food and feed applications. Another important factor influencing chickpea utilization in food products is the change in meal components, starch structure and their interaction during cooking/food processing procedures. Therefore, the results obtained in this study have predictive value only.

5.4.8 CONCLUSIONS

The kabuli and desi-type chickpea genotypes used in this study had similar total starch and amylose concentrations. In addition, the seeds of the two types of chickpea market classes did not differ in their starch granule sizes and/or amylopectin structure. Determination of starch hydrolysis of extracted starch showed that RDS was associated with HI, medium size starch granules, and starch concentration, RS was associated with amylose concentration and amount of very small size starch granules. SDS was closely associated with fine amylopectin structure. However, in seed meal starch hydrolytic activity determinations, the factors associated with RDS, SDS, HI and RS changed. This suggests that both starch characteristics and seed meal constituents affect chickpea utilization in food product development using whole chickpea seeds.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Chickpea is the third largest pulse crop in the world, which from the beginning of agriculture has been used for food and medicinal purposes (van der Masen, 1972). Most of the chickpea improvement has been focused to improve its agronomic performance and grain yield. Improvement of chickpea grain quality is difficult, because seed constituent analysis requires laboratory analysis, which is tedious and time consuming. In addition, deposition of seed storage constituents is strongly influenced by the environmental conditions and genetic factors (Wood and Grusak, 2007). Therefore, the first step towards grain quality improvement is to identify, and characterize seed constituents of interest, and study genotype by environment interaction for selected seed constituents of interest. This study focused on chickpea grain quality with the following objectives:

- Study variations, repeatability and genotype by environment interaction on thousand seed weight, starch, amylose and protein concentration of chickpea adapted to Saskatchewan's environment;
- Assess variations in global chickpea mini core for thousand grain weight, protein, starch and amylose concentrations;
- Characterize selected chickpea genotypes for their starch granule composition and structure and study their influence on starch hydrolysis, which is an important consideration for chickpea utilization for human nutrition and health benefits.

Several studies have been conducted on genotype by environment interaction in chickpea but not much information exist on effect of genotype by environment interaction on chickpea grain quality attributes such as starch, amylose, protein and thousand seed weight (Wood and Grusak, 2007). In this study, significant genotype, environment and genotype by environment interaction effects were detected for chickpea seed yield, thousand seed weight, starch, amylose and protein concentrations in both desi and kabuli varieties adapted to the western Canadian Prairies. However, protein concentration did not show genotype by environment interaction in kabuli class. In desi, seed yield ranged from 1120 (Myles) to 1710 (ICC-12512-9) $kgha^{-1}$. CDC Vanguard recorded the lowest protein ($185.4gkg^{-1}$) whilst Myles recorded the highest protein

(211.5gkg^{-1}) concentration. Myles had the lowest starch of 420.2gkg^{-1} whilst CDC Cabri had the highest starch concentration of 451.6gkg^{-1}. This finding is in agreement with values reported previously (Wood et al., 2008). CDC Cabri had the lowest amylose concentration of 263.8gkg^{-1} and CDC Anna recorded the highest amylose of 276.4gkg^{-1}. Total starch, amylose and protein showed less variation in desi. In general, amylose concentration in both desi and kabuli is lower than that reported (Wood et al., 2008; Chibbar et al., 2010; Hoover et al., 2010). We used SEC-HPLC method, which determines the actual amount of amylose molecules (Demeke et al., 1999), in debranched starch compared with iodine-based spectrophotometric methods that determine apparent amylose concentration.

In kabuli, overall seed yield and protein were comparatively lower than desi. However, kabuli varieties had higher total starch concentration than desi. This was not unexpected since kabuli seed size (8-10mm) was larger than desi (<8mm). Kabuli chickpea varieties had higher amylose concentration than desi varieties. Amylose concentration of 26.8-29.0 % reported in this study agrees with that of Singh et al. (1956) but inconsistent with 33.5 and 36.3 % reported for kabuli and desi by Saini and Knights (1984).

Positive and significant relationships between starch concentration and seed yield in desi and between starch concentration and seed weight in kabuli will offer plant breeders the possibility of indirect selection. However the negative relationship between starch and protein concentrations across chickpea market classes will require a compromise and indirect selection.

A principal component analysis (PCA) plot revealed CDC Anna to be the most stable and high yielding cultivar for both amylose and starch concentration in most environments while 316B-42 was the most stable and high-protein cultivar. Stability and yield of desi starch concentration was inconsistent when PCA plot result was compared with mean values across locations. This inconsistency was because PCA plots do not explain 100% of genotype and genotype by environment interaction (Yan, 2002). FLIP98-135C was a top yielder and most stable cultivar for amylose and starch concentration whilst Sandford was among the most stable for protein. PCA plots revealed that no single trait had a positive effect on seed quality in all environments. It will therefore not be feasible to improve quality by selecting for any specific trait in these varieties and environments.

Repeatability and heritability are genetic and phenotypic parameters required for efficient running of crop improvement programmes. Repeatabilities of all the traits were in a range of 0.12-0.94 (Desi r_{approx}) and 0.07-0.91 (Kabuli r_{approx}), being lower compared with heritabilities of 0.53-0.99 (Desi $H_{broad\ sense}$) and 0.36-0.97 (Kabuli $H_{broad\ sense}$). This trend disagrees with Falconer and Mackey (1996) proposition that repeatability sets upper limit to heritabilities in broad sense. Results are however supported by Dohm (2002) proposal that repeatability estimates may not set the upper limits to heritability if significant genotype by environment interaction is present.

The ICRISAT chickpea mini core collection of 211 accessions (209 accessions used in our study) reported to capture 70% of the useful genes in the entire core collection (Upadhaya and Ortiz, 2001) in addition to 12 advanced breeding lines from the Crop Development Centre of Canada and ICC15606 (desi), ICC3182, ICC32795 (all from India) were screened for amylose, total starch, protein, thousand seed weight, seed diameter and seed colour. Desi mini core mean concentrations of proteins, starch, amylose, seed colour, seed diameter and thousand seed weight were 26.2±1.3%, 51.2±2.2%, 27.6±1.2%, 33.8±6.2, 5.7±0.5mm and 158.1±31.6g, respectively. Interestingly, desi mini core starch and protein contents were the only quality traits that were higher than the 43.5% and 19.5%, respectively reported earlier in the varieties adapted to the western Canadian Prairies. This is an indication that desi mini core has the potential to be used for starch and protein improvement in chickpea. In kabuli, mean protein, starch, amylose, seed colour, seed diameter and thousand seed weight were 22.5±1.6%, 47.6±3.0%, 27.0±1.1%, 47.8±6.1, 6.8±0.5mm and 237.8±50.2g, respectively. Amylose concentration across mini core was comparable to 26.0-28.0% reported earlier in the varieties adapted to western Canada and elsewhere (Guillon and Champ, 2002). Almost all the accessions that had above average score seed diameter in both desi and kabuli also had above average score for thousand weight. This confirms the strong significant relationship between seed diameter and thousand seed weight in chickpea (Bicer, 2009). Both the extrinsic and intrinsic grain quality traits showed a high Shannon-Weaver Diversity index. This suggested that within the genotypes there was a high allelic diversity for the analyzed traits, therefore, detailed analysis can help to identify accessions of interest. However, the allelic diversity was limited to the narrow phenotypic diversity observed in this mini core collection. This emphasizes the need to develop a specialized mini core collection with large phenotypic diversity of grain quality traits.

In chickpea two distinct market classes, desi and kabuli are recognized. The desi has angular dark coloured seeds, while kabuli has owl-head shaped light coloured seeds. Seed constituent analysis did not reveal any difference between the two desi and kabuli-type chickpea. Starch digestibility is a major factor, affecting grain utilization. Compared to cereals, pulses are known to have less starch digestibility, and among pulses chickpea has one of the lowest rates of starch digestibility (Jenkins et al., 1980). Starch digestibility has been associated with starch amylose concentration. Elevated amylose diets are reported to have prebiotic effects on human beings as well as controlling obesity, cardiovascular diseases and colon cancer (Brown, 1996; Topping and Clifton, 2001). It is also reported that starch structure (amylose: amylopectin ratio, starch granule size and shape and amylopectin chain length distribution) affects its rate of hydrolysis (Shu et al., 2007; Tatsumi et al., 2007; Lee et al., 2008). For instance Stevnebo et al. (2006) reported that barley cultivars with low level of amylose had higher degree of starch hydrolysis than cultivars with normal and high amylose contents for all time intervals. In this study, rate of starch hydrolysis had no relationship with amylose concentration in chickpea. The reason could be that there was no difference between the amylose concentration of the accessions analyzed due to genetic bottlenecks.

Rate of starch hydrolysis of chickpea meal was higher than purified starch at all times (contrary to expected, Stevnebo et al. 2006) and the higher amount of α-amylase content of the meal could be the reason. However, chickpea has been reported to have slowly digestible starch concentration only second to that of mung bean among all the legumes (Sandhu and Lim, 2008). Rate of starch hydrolysis was relatively lower for both desi meal and pure starch when compared with kabuli meal and pure starch. The small magnitude of the significant positive correlation between amylose concentration and both slowly digestible starch and resistant starch after 4 hour of hydrolysis (r_{desi}=0.31, r_{kabuli}=0.28, $p<0.05$) between desi and kabuli could partly explain this. This trend could also be explained by differences in the amylose structure (Fitzgerald and Blanchard, 2005). Kabuli accessions had higher 5-20μm, 20-40 μm but lower 40-60 μm and 60-80 μm granule size volume percent than desi and that may account for the relatively slower rate of starch hydrolysis in desi genotypess. This observation supports the hypothesis that due to a higher surface area and lower crystallinity, small starch granules are degraded at a higher level than large granules (Stevnebo et al. 2006). Interestingly, accessions which had significantly

lower 60-80 µm granule volume percent had their granule size significantly affected by the environment.

The proportion of side-chains with DP<12 (A chains), DP13-25 (B1 chains), DP26-35 (B2 chains), and DP36-55 (C chains) in desi were in a range of 7.61-23.31%, 56.99-69.93%, 15.41-19.23%, 3.67-4.30%, respectively. In kabuli, side-chains with DP<12 (A chains), DP13-25 (B1 chains), DP26-35 (B2 chains), and DP36-55 (C chains) ranged from 7.97-20.39%, 57.09-69.50%, 16.52-18.62%, 3.91-5.41%, respectively. Two interesting groups of amylopectin structures emerged across chickpea types: FLIP97-133C (kabuli), ICC9848 (desi)-(group 1); and ICC 9862 (kabuli), ICC 2277 (kabuli), CDC Cabri (desi)-group 2). Group 1 and 2 were significantly different ($P<0.05$) from each other. Amylose had strong relationship with resistant starch in kabuli pure starch, desi pure starch, and desi meal starch when multivariate analysis was done. SDS, the most desirable form of dietary starch which completely but more slowly digest in the small intestine and attenuates postprandial plasma glucose and insulin levels (Jenkins et al. 1981), negatively correlated with HI in both pure starch and meal starch of desi and kabuli. The strong positive correlation between HI and RDS in kabuli corroborates the findings of Sandhu and Lim (2008) in chickpea. There was positive relationship between B2 chains of amylopectin and slowly digestible starch of pure starch and meal starch (except kabuli). However, B1 fraction of amylopectin chain length distribution significantly correlated with RS in both kabuli and desi meal starch as revealed by PCA. The low digestibility property of the studied chickpea genotypes may be attributed to the degree of crystalinity, enzyme inhibitors, soluble dietary fibre constituents and antinutrients such as polyphenols and phytic acid.

6.1 FUTURE RESEARCH DIRECTIONS

Since all carbohydrates are derived from simple sugars, reducing or increasing one carbohydrate component will affect others as a direct consequence of change in partitioning of sugars. If genetic variation cannot be found within existing germplasms, it will then be necessary to create it either by mutagenesis, introductions from gene banks especially current ICRISAT holdings, functional genomics strategies (Ganeshan et al., 2010) such as TILLING (Targeting Induced Local Lesions IN Genomes) (McCallum et al., 2000) or introduction of novel genes from other less commonly grown pulses (Chibbar et al., 2010). The degree of crystalinity, soluble dietary

fibre constituents and antinutrients such as polyphenols and phytic acid as they affect starch hydrolysis in these accessions must be investigated in the future. Chickpea starch biosynthetic enzymes should be profiled. This will allow for transcriptional characterization, an effort that can enhance the biochemical and molecular understanding of these enzymes towards a step in a direction of increasing starch quantity and quality during chickpea development. Future studies should also characterize in detail the environmental factors influencing and hampering quality improvement in chickpea. It will be of interest to study the changes in meal components, starch composition and structure during the food processing and cooking procedures to precisely evaluate the starch enzymatic hydrolysis potential of chickpea based food products.

6.2 CONCLUSIONS

- Variations occurring among seed composition of chickpea cultivars adapted to the western Canadian prairies was low.
- Significant genotype by environment interaction occurred for starch, amylose, protein (except for kabuli) concentrations, seed yield and thousand seed weight indicating that testing over a wide range of environments will be required.
- Repeatabilties of starch, amylose, and protein concentrations were low and inconsistent across chickpea market class.
- Broad sense heritability was higher than repeatability across all traits for all market classes implying that repeatability estimates does not set the upper limits to heritability if significant genotype by environment interaction is present.
- Negative relationship between seed constituents and yield indicates that selection for chickpea cultivars with desired seed composition may require compromise for yield and indirect selection.
- All the mini core accessions that had above average score seed diameter in both desi and kabuli also had above average score for thousand weight
- Amylose had strong relationship with resistant starch but not rate of starch hydrolysis.
- B1 fraction of amylopectin chain length distribution strongly influenced RS in both kabuli and desi meal starch.

LITERATURE CITED

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 44-16, 46-30, 54-21, 54-40A. The Association: St Paul, MN.

Abbo, S., A. Gopher, B. Rubin and S, Lev-Yadun. 2005. On the origin of Near Eastern founder crops and the 'dump-heap hypothesis'. Genetic Resources and Crop Evolution 52:491-495.

Abbo, S., J. Berger, and N.C. Turner. 2003. Evolution of cultivated chickpea: four bottlenecks limit diversity and contrain adaptation. Functional Plant Biology 30:1081-1087.

Abusin, S. A.E., A. B. Hassan and. E. E. Babiker. 2009. Nutritional evaluation of cooked Faba Bean (*Vicia faba* L.) And White Bean (*Phaseolus Vulgaris* L.) Cultivars. Australian Journal of Basic and Applied Sciences 3:2484-2490.

Agriculture and Agric-Food Canada. 2009. Irrigated Chickpea Trials at Canada Saskatchewan Irrigation Diversification Centre. http://www4.agr.gc.ca/AAFC-AAC/display-afficher.do?id=1190146757249&lang=eng Verified on July 25, 2009.

Aher, R.P., L.B. Mhase, M.R. Manjare, and T.B. Kolte. 1998. Phenotypic stability for grain yield in chickpea under irrigated condition. Indian Journal of Pulses Research 11:115-116.

Al-karaki, G.N. and K.I. Ereifej. 1997. Chemical composition of pea seeds as related to seed yield under arid and semiarid Mediterranean environments. Journal Agronomy and Crop Sciences 178:97-102.

Alwawi, H., M. Moulla and W. Choumane. 2010. Genotype-environment interaction and genetic parameters in chickpea (*Cicer arietinum* L.) Landraces. Journal of Agricultural Science 2:154-161.

Anbessa, Y., T.D. Warkentin, A. Vandenberg and R. Ball. 2006. Inheritance of time to flowering in chickpea in a short season temperate environment. Journal of Heredity 97: 55-61.

Anon. 1987. Measurement of the starch contents of commercial starches. Starch/Starke 39:414-416.

Arshad, M., A. Bakhsh, A.M. Haqqani and M. Bashir. 2003. Genotype-environment interaction for grain yield in chickpea (Cicer arietinum L.). Pakistan Journal of Botany 35:181-186.

Atta, B.M.,. T. M. Shah, G. Abbas and. M. A. Haq. 2009. Genotype x environment interaction for seed yield in kabuli chickpea (*Cicer arietinum* L.) genotypes developed through mutation breeding. Pakistan Journal of Botany 41:1883-1890

Attia, R.S., A.M.E. Shehata, M.E. Aman and M.A. Hamza. 1994. Effect of cooking and decortication on the physical properties, the chemical composition and the nutritive value of chickpea (*Cicer arietinum* L.). Food Chemistry 50:125-131.

Bakhsh, A., M. Arshad and A.M. Haqqani. 2006. Effect of genotype x environment interaction on relationship between seed yield and its components in chickpea (*Cicer arietinum* L.). Pakistan Journal of Botany 38:683-690.

Banik, B.R., R. Sarker and D.B. Pandit. 1997. Genotype environment interaction of seed yield and seed weight in chickpea. Legume Research 20:230-232.

Banks, W. and C. T. Greenwood. 1975. Starch and its components. Edinburgh: Edinburgh University Press. pp. 342.

Bar-Yosef, O.1998. The Natufian culture in the Levant, threshold to the origins of agriculture. Evolutionary Anthropology 6:159-177.

Baroja-Fernandez, E., J.F. Munoz, A. Zandueta-Criado, T.M. Moran-Zorzano, A.M. Viale, N. Alonso-Casajus and J. Pozueta-Romero. 2004. Most of ADP-glucose linked to starch

biosynthesis occurs outside the chloroplast in source leaves. Proceedings of National Academy of Sciences (USA) 101:13080-13085.

Basford, K.E and M. Cooper. 1998. Genotype x environment interactions and some considerations of their implications for wheat breeding in Australia. Australian Journal of Agricultural Research 49:153-174.

Becker, W. A. 1984. Manual of Quantitative Genetics 4th edition, Academic Enterprises, Pullman, Washington, pp.188.Behall, K.M. and J.C. Howe. 1995**.** Contribution of fiber and resistant starch to metabolizable energy. American Journal of Clinical Nutrition 62:1158S-1160S.

Bello-Pe´rez, L. A., J. J. Islas-Herna´ndez, J. R. Rendo´n-Villalobos, E. Agama-Acevedo, L. Morales-Franco and J. Tovar. 2007. *In vitro* starch digestibility of fresh and sun-dried faba beans (*Vicia faba* L.). Journal of the Science of Food and Agriculture 87:1517–1522.

Berger, J., J.A. Palta, C. Ludwig, D. Shrestha, M.C. Mackay, K.A. Street, J. Konopka, S. Jenkins, K.N. Adhinkari, H.C. Clarke, J.S. Sandhu and H. Nayyar. 2008. Emerging opportunities for agriculture: investigating plant adaptation by characterizing germplasm collection habits. Proceedings of the 14th Australian Agronomy Conference, September 2008. (ed. Unkovich, M.J.) .

Berger, J.D., J. Speijers, R.L. Sapra and U.C. Sood. 2007. Genotype by environment interaction and chickpea improvement, in Chickpea Breeding and Management, ed. by Yadav SS, Redden R, ChenW and Sharma B. CABI International, Wallingford, pp. 617–629.

Berger, J.D., M. Ali, P.S. Basu, B.D. Chaudhary, S.K. Chaturbedi, P.S. Desmuk, P.S. Dharmaraj, S.K. Dwivedi, G.C. Gangadhar, P.M. Guar, J. Kumar, R.K. Pannu, K.H.M. Siddique, D.N. Singh, D.P. Singh, S.J. Singh, N.C. Turner, H.S. Yadava and S.S. Yadav. 2006. Genotype by environment studies demonstrate the critical role of phenology in adaptation of chickpea (*Cicer arietinum* L.) to high and low yielding environments of India. Field Crops Research 98: 230-244.

Berger, J.D., N.C. Turner, K.H.M. Siddique, E.J. Knights, R.B. Brinsmead, I. Mock, C. Edmondson and T.N. Khan. 2004. Genotype by environment studies across Australia reveal the importance of phenology for chickpea (*Cicer arietinum* L.) improvement. Australian Journal of Agricultural Research 55:1-14.

Berger, J.D., S. Abbo and C. Turner. 2003. Ecogeography of annual wild Cicer species: the poor state of the world collection. Crop Science 43:1076-1090.

Berry, C.S. 1986. Resistant starch: formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. Journal of Cereal Science 4: 301-314.

Bertozo, M.R. and J.F.M. Valls. 2001. Seed storage protein electrophoresis in *Arachis pintoi* and *A. repens* (*Leguminosae*) for evaluating genetic diversity. Genetic Resources and Crop Evolution 48:121-130.

Bhattacharjee, R., I. S. Khairwal, P. J. Bramet and K. N. Reddy. 2007. Establishment of a pearl millet [*Pennisetum glaucum* (L.) R. Br.] core collection based on geographical distribution and quantitative traits. Euphytica 155:35-45.

Biçer, B. T. 2009. The effect of seed size on yield and yield components of chickpea and lentil. African Journal of Biotechnology 8:1482-1487.

Binghams, S.A. 1990. Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. Proceedings of the Nutrition Society 49:153-171.

Boag, P.T. 1983. The heritability of external morphology in Darwin's ground finches (*Geospiza*) on Isla Daphne Major, Galapagos. Evolution 37:877-894.

Boake, C.R.B. 1989. Repeatability: its role in evolutionary studies of mating behavior. Evolutionary Ecology 3:173-182.

Broberg, S., K. Koch, R. Andersson and L. Kenne. 2000. A comparison between MALDI-TOF mass spectrometry and HPAEC-PAD analysis of debranched starch. Carbohydrate Polymers 43:285-289.

Brown, A.H.D. 1995. The core collection at the crossroads. In: Hodkin, T., A.H.D. Brown, J.L. Van Hintum. and E.A.V. Morales (eds.), Core Collections for Today and Tommorrow, International Plant Genetics Resources Institute (IPGRI), Rome, Italy, Wiley-Ssayce Publication pp.3-19.

Brown, A.H.D. and B.S. Weir 1983. Measuring genetic variability in plant populations. Pp 219-283. In: Isozymes in plant genetics and breeding. Part A. Eds. A. Tanksley and T.J. Orton. Elsevier, Amsterdam, Netherlands.

Brown, I. 1996. Complex carbohydrates and resistant starch. Nutrition Reviews 54: S115-S119.

Brown, A.H.D. 1989. Core collection: a practical approach to genetic resources management. Genome 31:818-824.

Buchanan, B.B., W. Guissem and R.L. Jones. 2005. Biochemistry and Molecular Biology of Plants. 5th ed., Macmillan, U.S.A. pp 630-675.

Carlyle, W.J. 2004. The rise of specialty crops in Saskatchewan, 1981-2001. The Canadian Geographer 48:137-151.

Carré, B., J. P. Melcion, J. L. Widiez, and P. Biot. 1998. Effects of various processes of fractionation, grinding and storage of peas on the digestibility of pea starch in chickens. Animal Feed Science Technology 71:19–33.

Champ, M. 1992. Determination of resistant starch in foods and food products: interlabolatory study. European Journal of Clinical Nutrition 46: S51-S62.

Champ, M., A-M., Langkilde, F. Brouns, B. Kettilz and Y. Le Bail-Collet. 2003. Advances in dietary fibre characterization. 2. Consumption, chemistry, physiology and measurement of resistant starch; implications for health and food labelling. Nutrition Research 16:143-161.

Chandra, S., M.S. Sohoo and K.B. Singh. 1971. Genotype x environment interaction for yield in gram. Journal of Research Punjab Agricultural University 8:165-168.

Chibbar, R.N., P. Ambigaipalan and R. Hoover. 2010. Molecular diversity in pulse seed starch and complex carbohydrates and its role in human nutrition and health. Cereal Chemistry 87:342-352.

Chibbar, R.N., S. Ganeshan and M. Båga. 2007. In plants novel starch synthesis. In: Improvement of Crop plants for Industrial Uses. P. Ranalli (ed.) Springer Dordrecht, Netherlands. pp 181-208.

Chibbar, R.N., S. Ganeshan, M. Båga and R.L. Khandelwal. 2004. Carbohydrate metabolism. In: Encyclopedia of Seed Science (1). C. Wrigley., H. Corke and C. Walker (eds). Elsevier Academic Press, Oxford, pp. 168–179.

Chibbar, R.N. and M. Båga 2003. Genetic modification of primary metabolism: carbohydrates. In: Encyclopedia of Applied Plant Sciences (1). B. Thomas, D.J. Murphy and B.G. Murray (eds). Elsevier Academic Press, Oxford, pp. 449–459.

Chung, H., Q. Lui, R. Hoover, T.D. Warkentin and B. Vandenberg. 2008. In vitro starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. Food Chemistry 11: 316-321.

Croy, R.R.D. and J.A. Gatehouse. 1985. Genetic engineering of seed proteins: current and potential application. In: Dodds JH (ed.) Plant Genetic Engineering. Cambridge: Cambridge University Press, pp. 143–268.

Dahiya, B.S., I.S. Solanki and R. Kumar. 1985. Germination rate and its genetics in chickpea. International Chickpea Newsletter 13:6-8.

De Almeida Costa, G.E., K. da Silva Queiroz-Monci, S.M.P.M. Reis and A.C. de Oliveira. 2006. Chemical composition,dietary fibre and resistant starch contents of rawand cooked pea, common bean, chickpea and lentil legumes. Food Chemistry 9:327–330.

de Fekete, M.A.G, L.F. Leloir, and C.E. Cardini. 1960. Mechanism of starch biosynthesis. Nature 187:918-919.

Dehghani, H., H. S. Sabaghpour and A. Ebadi. 2010. Study of Genotype x Environment Interaction for Chickpea Yield in Iran. Agronomy Journal 102:1-8.

DeLacy, I.H., K.E. Basford, M. Cooper, J.K. Bull and C.G. McLaren. 1996. Aanalysis of multi-environment trials-An historical perspective. In: M. Cooper and G.L. Hammer (eds). Plant Adaptation and Crop Improvement. CAB International, Wallingford, UK, pp39-124.

Delwiche, S.R., M.M. Bean, R.E. Miller, B.D. Webb and P.C. Williams. 1995. Apparent amylose content of milled rice by near-infrared reflectance spectrophotometry. Cereal Chemistry 72:182-187.

Demeke, T., P. Hucl, E. Abdel Aal, M. Båga, R.N. Chibbar. 1999. Characterization of wheat waxy A protein and its effect on starch properties. Cereal Chemistry 76:694-698.

Demeke, T., P. Hucl, R.B. Nair, T. Nakamura and R. N. Chibbar. 1997. Evaluation of Canadian and other wheats for waxy proteins. Cereal Chemistry 74:442-444.

Denyer, K., P. Johnson, S. Zeeman and A.M. Smith. 2001. The control of amylose synthesis. Journal of Plant Physiology 158: 479-487.

Deshmukh, R.B., N.S. Kute, R.P. Aher and L.B. Mhase. 1998. Stability for grain yield in chickpea. Indian Journal of Pulses Research 11:79-81.

Dohm, R.M. 2002. Repeatability estimates do not always set an upper limit to heritability. Functional Ecology 16:273–280.

Duke, J.A. 1981. Handbook of legumes of world economic importance. Plenum Press, New York. pp. 52-57.

Eisemann, R.L., M. Cooper and D.R. Woodruff. 1990. Beyond the analytical methodology-Better interpretation of genotype-by-environment interaction. In: M.S. Kang (ed) Genotype-by-environment Interaction and Plant Breeding. Louisiana State University, Baton Rouge, Louisiana, pp. 108-117.

Emami, S., L.G. Tabil and R.T. Tyler. 2006. Isolation of starch and protein from chickpea flour using a hydrocyclone and isoelectric precipitation method. American Society of Agricultural and Biological Engineers (ASABE). Paper No.: 066090, 2050 Niles Road, St. Joseph, MI 49085-9659 USA.

Emami, S. and L.G. Tabil. 2002. Processing of starch-rich and protein-rich fractions from chickpeas- a review. American Society of Agricultural and Biological Engineers. Paper No. MBSK 02-212, ASAE, 2050 Niles Road, St. Joseph, MI 49085-9659 USA.

Englyst, K.N., H.N. Englyst, G.J. Hudson, T.J. Cole and J.H. Cummings. 1992a. Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. American Journal of Clinical Nutrition 69:448-454.
Englyst, H.N., S.M. Kingman and J.H. Cummings. 1992b. Classification and measurement of nutritionally important starch fraction. European Journal of Clinical Nutrition 46:S33-S50.
Englyst, H.N. and J.H. Cummings. 1990. Non-starch polysaccharides (dietary fibre) and resistant starch. In: I. Furda and C.J. Brine (eds). New developments in dietary fibre. New York: Plenum Press. pp. 205-225.
Englyst, H.N, H.S. Wiggins and Cummings, J.H. 1982. Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 107:307-318.
Ereifej, K.I., G.N. Al-Karaki and M.K. Hammouri. 2001. Seed chemical composition of improved chickpea cultivars grown under semiarid Mediterranean conditions. International Journal of Food Properties 4:239–246.
Falconer, D.S. and T.F. Mackay. 1996. Introduction to Quantitative Genetics, 4th edn. Longman, Harlow.
Fernando, R.L., S.A. Knights and D. Gianola. 1984. On a method of estimating the genetic the genetic correlation between characters measured in different experimental units. Theoretical and Applied Genetics 67:175-178.
Findlay, C.S. and F. Cooke. 1983. Genetic and environmental components of clutch size variance in a wild population of lesser snow geese (*Anser caerulescens caerulescens*). Evolution 37:724-734.
Fitzgerald, M. and C. Blanchard. 2005. Understanding Amylose Structure, What It Controls and What Controls It. http://hdl.handle.net/2123/136. Verified on September 18, 2009.
Food and Agriculture Organization. 2009. World Chickpea Production Data, FAOSTAT Database, Food and Agriculture Organization of the United Nations. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor Last verified: January 20, 2010.
FAO, Agriculture production statistics. [Online]. 2005. Available: http://faostat.fao.org/default.aspx [Last accessed: 2 June 2006].
Frankel, O.H.1984. Genetic perspective of germplasm conservation. In: W. Arber et al. (ed) Genetic Manipulations: Impact on Man and Society. Cambridge Univ. Press, Cambridge,UK, pp. 161-170.
Fredriksson, H., J. Silverio, R. Anderson, A.C. Eliasson and P. Aman. 1998. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. Carbohydrate Polymers 35:119-134.
Gallant, D.J., B. Bouchet and P.M. Baldwin. 1997. Microscopy of starch: evidence of a new level of granule organization. Carbohydrate Polymers 32: 177-191.
Gallant, D.J., B. Bouchet, A. Buleon and S. Perez. 1992. Physical characteristics of starch granules and susceptibility to enzymatic degradation. European Journal of Clinical Nutrition 46:3-16.
Gan, Y., B.D. Gossen, L. Li, F. Greg and S. Banniza. 2007. Cultivar type, plant population and *aschochyta* blight in chickpea. Agronomy Journal 99:1463-1470.

Ganeshan, S., P. Sharma and R.N. Chibbar 2010. Functional genomics for crop improvement. In: Jain, S.M. and D.S. Brar (eds.) Molecular techniques for crop improvement. Springer New York, N.Y. USA. Pp 63-95.

Genkina, N.K., V.I. Kiseleva and T. Noda. 2009. Comparative investigation on acid hydrolysis of sweet potato starches with different amylopectin chain-length. Starch/Starke 61:321-325.

Geervani, P. 1991. Utilization of chickpea in India and scope for novel and alternative uses. p. 47-54. In: Uses of Tropical Grain Legumes: Proceedings of Consultants' Meeting, 27-30 March, 1989. ICRISAT Center, Patancheru, Andhra Pradesh, India.

Gibson, T.S., V.A. Solah and B.V. McCleary. 1997. A procedure to measure amylose in cereal starches and flours with concanavalin A. Journal of Cereal Science 25:111-119.

Gitzelman, P. and S. Auricchio. 1965: The handling of soya alpha-galactosides by a normal and a galactosemic child. Pediatrics 36: 231 – 235.

Goni, I., L. Garcia-Diz, and F. Saura-Calixto. 1997. A starch hydrolysis procedure to estimate glycemic index. Nutrition Research 17:427-437.

Goni, I., E. Garcia-Diz, E. Manas and F. SauraCalixto. 1996. Analysis of resistant starch: a method for foods and food products. Food Chemistry 56:445-449.

Guillon, F. and M.M.J. Champ. 2002. Carbohydrate fractions of legumes: uses in human nutrition and potential for health. British Journal of Nutrition 88:S293-S306.

Hanashiro, I., K. Itoh, Y. Kuratomi, M. Yamazaki, T. Igarashi, J. Matsugasako and Y. Takeda. 2008. Granule-Bound Starch Synthase I is Responsible for Biosynthesis of Extra-Long Unit Chains of Amylopectin in Rice. Plant and Cell Physiology 49:925-933.

Hanashiro, I., J. Abe and S. Hizukuri. 1996. A periodic distribution of the chain length of amylopectin as revealed by high performance anion-exchange chromatography. Carbohydrate Research 283:151-159.

Hardy, M.R. and R.R. Townsend. 1988. Separation of positional isomers of oligosaccharides and glycopeptides by high performance anion-exchange chromatography with pulsed amperometric detection. Proceedings of National Academy of Sciences (USA) 85:3289-3293.

Hedley, C. 2001. Strategies for manipulating grain legume carbohydrates. In: Hedley, C.L. (ed.). Carbohydrates in grain legume seeds, improving nutritional quality and agronomic characteristics. CABI Publishing, N.Y. pp. 322.

Hildebrand, P.E. 1990. Modified stability analysis and on farm research to breed specific adaptability for ecological diversity. In: Kang, M.S. (ed.) Genotype by environment interaction and plant breeding. Louisiana State University, Baton Rouge, LA, USA pp 169-180.

Hizukuri, S. 1986. Polymodal distribution of the chain lengths of amylopectins, and its significance. Carbohydrate Research 147:342-347.

Hoover, R., T. Hughes, H.J. Chung and Q. Liu 2010. Composition, molecular structure, properties and modification of pulse starches – a review. Food Research International 43: 399-413.

Hoover, R. and W. S. Ratnayake. 2002. Starch characteristics of black bean, chickpea, lentil, navy bean and pinto bean cultivars grown in Canada. Food Chemistry 78:489–498.

Hoover, R. and F.W. Sosulski. 1990. Composition, structure, functionality and chemical modification of legumes starches: A review. Canadian Journal of Pharmacology 69:79-92.

Horbowicz, M. and R.L. Obendorf. 1994. Seed desiccation tolerance and storability. Dependence on flatulence-producing oligosaccharides and cyclitols-review and survey. Seed Science Research 4:385–404.
Horton, P. 2000. Prospects for crop improvement through the genetic manipulation of photosynthesis: Morphological and biochemical aspects of light capture. Journal of Experimental Botany 51:475-485.
Hucl, P. and R.N. Chibbar. 1996. Variation for starch concentration in spring wheat and its repeatability relative to protein concentration. Cereal Chemistry 73:756–758.
Hylla, S., A. Grostner, G. Dusel, H. Anger, H.P. Bartram, S.U. Christl, H. Kasper and W. Scheppach. 1998. Effect of resistant starch on the colon in healthy volunteers. American Journal of Clinical Nutrition 676:136-142.
Ibanez, M.V., F. Rincon, M. Amaro and B. Martinez. 1998. Intrinsic variability of mineral composition of chickpea (*Cicer arietinum* L.). Food Chemistry 63:55-60.
Ibrikci, H., S.J.B. Knewston and M. Grusak. 2003. Chickpea leaves as a vegetable green for humans: evaluation of mineral composition. Journal of the Science of Food and Agriculture 83:945-950.
IBPGR, ICRISAT and ICARDA, 1993. Descriptors for chickpea (*Cicer arietinum* L.). International Board for Plant Genetic Resources, Rome, Italy; International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India; International Centre for Agricultural Research in the Dry Areas. Aleppo, Syria.
International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). 2007. Crops: Chickpea. http://test1.icrisat.org/ChickPea/Chickpea.htm. Verified on December 20, 2007.
Inouchi, N., H. Ando, M. Asaoka, K. Okuno, H. Fuwa. 2000. The effect of environmental temperature on distribution of unit chains of rice amylopectin. Starch/Starke 52:S8-12.
Iruela, M., J. Rubio, J.I. Cubero, J. Gil and T. Millan. 2002. Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. Theoretical and Applied Genetics 104:643-651.
Jain, K.C., B.P Pandya and K. Pande. 1984. Stability of yield components of chickpea genotypes. Indian Journal of Genetics 44:159-163.
Jambunathan, R. and U. Singh. 1980. Studies of desi and kabuli chickpea (*Cicer arietinum* L.) cultivars. Chemical composition. International Workshop on Chickpea Improvement, ICRISAT, Hyderabad, India, pp. 61-66.
Jaroslav, B. 2009. Role of amylose in structure-function relationship in starches from Australian wheat varieties. PhD Thesis. Faculty of Agriculture, Food and Natural Resources. University of Sydney. pp. 225.
Jenkins, D.J., T.M. Wolever, A.L. Jenkins, M.J. Thorne, R. Lee, J. Kalmusky, Reichert, R. and Wong, G.S. 1983. The glycaemic index of foods tested in diabetic patients: a new basis for carbohydrate exchange favouring the use of legumes. Diabetologia 24:257–264.
Jenkins, D.J.A., T.M.S. Wolver, R.H. Taylor, H. Barker, H. Fielder, J. M Baldwin, A. C. Bowling, H. C. Newman, A. L. Jenkins and D. V. Goff. 1981. Glycemic index of foods: A physiological basis for carbohydrate exchange. American Journal of Clinical Nutrition 34:362-366.
Jenkins, D.J.A., T.M.S. Wolver, R.H. Taylor, H. Barker, H. Fielder. 1980. Exceptionally low blood glucose responses to dried beans, comparison with other carbohydrate foods. British Medical Journal 281:578-580.

Kareem, Z., S. Lev-Yadun, A. Gopher, P. Weinberg and S. Abbo. 2007. Chickpea domestication in the Neolithic Levant through the nutritional perspective. Journal of Archaeological Science 34:1289-1293.

Katiyar, P.K., L. Ram and R.P. Katiyar. 1992. Phenotypic stability of advance generation populations of chickpea for yield and its components. Indian Journal of Genetics and Plant Breeding 52:341-346.

Kays, S. E., N. Shimizu, F.E. Barton II., and K. Ohtsubo. 2005. Near-Infrared Transmission and Reflectance Spectroscopy for the Determination of Dietary Fiber in Barley Cultivars. Crop Science 45:2307–2311.

Khan, I.A., B.A. Malik and M. Bashir. 1988. Investigation of genotype x environment interaction for seed yield in chickpea (*Cicer arietinum* L.). Pakistan Journal of Botany 20:201-204.

Khorgade, P.W., V.B. Rathi, M.N. Narkhede and B.A. Sakhare. 2000. Genotype x environment interaction and stability parameters in chickpea. Annals of Plant Physiology 14:32-35.

Knights, E.J., N. Acikg¨ oz, T.W. Warkentin, G. Bejiga, S.S. Yadav, and J.S. Sandhu. 2007. Area, production and distribution. In: Chickpea Breeding and Management. S.S. Yadav, R. Redden, W. Chen and B. Sharma (eds). CABI International, Wallingford, pp. 167–178.

Knights, E.J. and R.J. Mailer. 1989. Association of seed type and colour with establishment, yield and seed quality in chickpea (*Cicer arietinum*). Journal of Agricultural Science 113:325–330.

Knights, E. J.1980. Kabuli-Desi introgression: the experience in Australia. In 'Proceedings International Workshop on Chickpea Improvement'. Hyderabad, India, 1979. pp. 70-4.

Koizumi, K., M. Fukuda and S. Hizukuri. 1991. Estimation of the distribution of chain length of amylopectins by high-performance liquid chromatography with pulsed amperometric detection. Journal of Chromatography 585:233-238.

Kozlowska, H., P. Aranda, J. Dostalova, J. Frias, M. Lopez-Jurado, J. Pokorny, G. Urbano, C. Vidal-Valverde and Z. Zdyunczyk. 2001. Nutrition. In: Hedley, C.L. (ed.). Carbohydrates in grain legume seeds, improving nutritional quality and agronomic characteristics. CABI Publishing, N.Y. pp. 322.

Krochko, J.E. and J.D. Bewley. 2000. Seed storage proteins in cultivars and subspecies of alfalfa (*Medicago sativa* L.). Seed Science Research 10:423–434.

Kumar, J., K.B. Singh, R.S. Malhotra, J.H. Miranda and G.T. Das. 1996. Genotype x environment interaction for seed yield in chickpea. Indian Journal of Genetics and Plant Breeding 56:69-78.

Kumar, S., O. Singh, H.A. Van Rheenen, and KV.S. Rao. 1998. Repeatability of different stability parameters for grain yield in chickpea. Plant Breeding 117:143-146.

Ladizinsky, G. 1975. A new *Cicer* from Turkey. Notes of the Royal Botanic Garden Edinburgh 34: 201-202.

Lee, C., Q. Le, Y. Kim, J. Shim, S. Lee, J. Park, K. Lee, S. Song, J.H. Auh, S. Lee, and K. Park. 2008. Enzymatic synthesis and properties of highly branched rice starch amylose and amylopectin cluster. Journal of Agriculture and Food Chemistry 56:126-131.

Lessells, C.M. and P.T. Boag. 1987. Unrepeatable repeatabilities: a common mistake. Auk 104:16–121.

Liljeberg, H. and I. Bjorck 1994. Bioavailability of starch in bread products. European Journal of Clinical Nutrition 48:151-163.

Lindeboom, N., P.R. Chang and R.T. Tyler. 2004. Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. Starch/Starke 56:89-99.

Lineback, D. R. and C. H. Ke. 1975. Starches and low molecular weight carbohydrates from chickpea and horse gram flours. Cereal Chemistry 52: 334–347.

Lui, S., J. E. Manson, M.J. Stampfer, M.D. Holmes, F.B. Hu, S.E. Hankinson and W.C. Willett. 2001. Dietary glycemic load assessed by food frequency questionnaire in relation to plasma high-density-lipoprotein cholesterol and fasting plasma triacylglycerols. American Journal of Clinical Nutrition 73:560-566.

Madhusudan, B. and R.N. Tharanathan 1996. Structural studies of linear and branched fractions of chickpea and finger millet starches. Carbohydrate Research 284:101-109.

Madhusudan, B. and R.N. Tharanathan 1995. Legume and cereal starch-why difference in digestibility? Part I. Isolation and composition of legume (green ram and Bengal gram) starches. Starch/Starke 47:165-171.

Malhotra, R.S. and K.B. Singh. 1991. Classification of chickpea growing environments to control genotype by environment interaction. Euphytica 58:5-12.

Matsui, H., T. Senoura, N. Isono, Y. Sakurai, S. Hamada, H. Ito. 2003 Granule bound starch synthase I isozyme localized predominantly in kidney bean leaves. Journal of Applied Glycosciences 50:493–497.

McCallum, C.M., L. Comai, E.A. Greene and S. Henikoff. 2000. Targetting induced local lesions in genomes (TILLING) for plant functional genomics. Plant Physiology 123:439.

McCleary, B.V. and D.A. Monaghan. 2002. Measurement of resistant starch. Journal of AOAC International 85: 665-675.

McClearly, B. V., V. Solah, and T.S. Gibson. 1994. Quantitative measurement of total starch in cereal flours and products. Journal of Cereal Science 20: 51-58.

McVicar R, P. Pearse, K. Panchuk, C. Brenzil, S. Hartley, J. Yasinowski, C. Harris, D. Goodwillie, T. Warkentin and S. Banniza. Chickpea in Saskatchewan. [Online]. (2002). Available: http:// www.agr.gov.sk.ca/docs/crops/pulses/production information/ chickpea2002.asp [Last accessed: 22 July 2006].

Mehra, R.B., P.N. Bahl and A.N. Pahuja 1980. Phenotypic stability and adaptability of newly developed varieties of chickpea in Northern India. India Journal of Agricultural Science 50:218-222.

Mendosa, D. 2009. Living with diabètes. http://www.mendosa.com/chanadal.html. , Verified online on July 27, 2009.

Mestres, C., F. Matencio, B. Pons, M. Yajid, and G. Fliede. 1996. A Rapid Method for the Determination of Amylose Content by Using Differential-Scanning Calorimetry Starch/Starke 48:S.2-6.

Mhase, L.B., M.R. Manjare, R.P. Aher and T.B. Kolte. 1998. Stability of grain yield in chickpea under rain fed condition. Indian Journal of Pulses Research 11:120-122.

Miao, M., T. Zhang, W. Mu and B. Jiang. 2009. Characteristics of kabuli and desi chickpea starches cultivated in China. Food Chemistry 113:1025-1032.

Michalskaa, J., H. Zaubera, B. B. Buchanana, F. J. Cejudoc and P. Geigenbergera. 2009. NTRC links built-in thioredoxin to light and sucrose in regulating starch synthesis in chloroplasts and amyloplasts. Proceedings of the National Academy of Sciences 106:9908-9913.

Mikulikova, D. and J. Kraic 2006. Natural sources of health-promoting starch. Journal of Food and Nutrition 45:69-76.

Milligan, G.W. and M. Cooper. 1985. An examination of procedures for determining the number of clusters in a data set. Psychometrika 50:159-179.

Mitchell, G.A. 1990. Methods of starch analysis. Starch/Starke 42:131-134.

Moreno, M. and J.I. Cubero. 1978. Variation in *Cicer arietinum* L. Euphytica 27:465-485.

Morell, M.K. and A.M. Myers. 2005. Towards the rational design of cereal starches. Current Opinion in Plant Biology 8:204–210.

Morell, M.K., C. Konik-Rose, R. Ahmed, Z. Li, and S. Rahman. 2004. Synthesis of Resistance Starch in Plants. Association of Official Analytical Chemists (AOAC) International 87:740-748.

Morell, M.K., M.S. Samuel, M.G. O'Shea. 1998. Analysis of starch structure using fluorophore-assisted carbohydrate electrophoresis. Electrophoresis 19:2603-2611.

Morris, C.F. 2004. Seed quality attributes. In: Encyclopedia of Seed Science (1) C. Wrigley., H. Corke and C. Walker (eds). Elsevier Academic Press, Oxford, pp. 238–254.

Mortensen, P.B. and M.R. Clausen. 1996. Short-chain fatty acids in the human colon. Scand. J. Gastroenterol 31:132-148.

Muehlbauer, F.J., and A. Tullu. 1997. *Cicer arietinum* L. NewCROP FactSHEET. Center for New Crops and Plant Products. Purdue University, West Lafayette, IN. http://www.hort.purdue.edu/newcrop/cropfactsheets/Chickpea.html.

Muir, J.G and K. O'Dea. 1992. Measurement of resistant starch: factors affecting the amount of starch escaping digestion in vitro. American Journal of Clinical Nutrition 56:123-127.

Mukerjea, R. and J F. Robyt. 2005a. Starch biosynthesis: the primer nonreducing-end mechanism versus the nonprimer reducing-end two-site insertion mechanism. Carbohydrate Research 340: 245-255.

Mukerjea, R. and J.F. Robyt. 2005b. Starch biosynthesis: further evidence against the primer nonreducing-end mechanism and evidence for the reducing-end two-site insertion mechanism. Carbohydrate Research 340:2206-2211.

Mukerjea, R., L. Yu and J F. Robyt. 2002. Starch biosynthesis: mechanism for the elongation of starch chains. Carbohydrate Research 337:1015-1022.

Muller, H. L., R. M. Kirchgessne and F. X. Roth. 1989. Energy utilization of intracaecally infused carbohydrates and casein in sows. In: Energy Metabolism of Farm Animals. pp. 123–126, Y. Van der Honning and W. H. Close (eds)., Pudoc, Wageningen.

Munier-Jolain, N.G and B. Ney. 1995. Variability of seed growth rate of various pea cultivars (*Pisum sativum* L.) in relation to their seed cell number in improving production and utilization of grain legumes. Proceedings of the second European Conference on Grain Legumes, Copenhagen, pp. 32-34.

Narayanan, A., N.P Saxena and A.R. Sheldrake. 1981. Varietal differences in seed size and seedling growth of pigeon pea and chickpea. Indian Journal of Agricultural Science. 51:389-393.

Nestares, T., M. Lopez-Frais, M. Barrionuevo and G. Urbano. 1996. Nutritional assessment of raw and processed chickpea (*Cicer arietinum* L.) protein in growing rats. Journal of Agricultural and Food Chemistry 44:2760-2765.

Nleya, T.M., G.C. Arganosa, A. Vandenberg, and R.T. Tyler. 2002. Genotype and environment effect on canning quality of kabuli chickpea. Canadian Journal of Plant Sciences 82:267–272.

O'Shea, M.G. and M.K. Morell 1996. High resolution slab gel electrophoresis of 8-amino- 1,3,6-pyrenetrisulfonic acid (APTS) tagged oligosaccharides using a DNA sequencer. Electrophoresis 17:681-688.

Pande, S., K.H.M. Siddique, G.K. Kishore, B. Baaya, P.M. Gaur, C.L.L. Gowda, T. Bretag, J.H. Crouch 2005. Aschochyta blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity and disease management. Australian Journal of Agricultural Research 56:317-332.

Pennock, D. 2006. Taxonomic Classification of Saskatchewan Soils. Verified online on September 19, 2008 at http://www.soilsofsask.ca.

Pereira, M.A., D.R. Jacobs, J.J. Pins, S.K. Raatz, M.D. Gross, J.L. Slavin and E.R. Seaquist. 2002. Effect of whole grains on insulin sensitivity in overweight hyperinsulinaemic adults. American Journal of Clinical Nutrition 75:848-855.

Peterbauer, T. and A. Richter. 2001. Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Science Research 11: 185-197.

Phillips, J., J.G. Muir, A. Birkett, Lu, Z.X., G.P. Jones and K. O'Dea. 1995. Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. American Journal of Clinical Nutrition 61:1-10.

Popalghat, G.R., J.V. Patil, R.B. Deshmukh and L.B. Mhase. 1999. Stability for yield and yield components in chickpea (*Cicer arietinum* L.). Legume Research 22:254-258.

Popov, M.G. 1929. The genus *Cicer* and its species. Trudy Prikladnoi Botanike Genetike I Selekcii 21:1-254.

Potokina, E. and E. Eggi. 1997. Protein portraits of *Vicia* L. species. Seed protein electrophoresis in taxonomical study of *Vicia* L. Haussknechtia no.7: 40.

Preiss, J. and M.N. Sivak. 1996. Starch synthesis in sinks and sources. In: Zamski, E., Shaffer, A.A (eds). Photoassimilate distribution and partitioning in plants and crops: source-sink relationships. New York: Marcel Dekker 63-96.

PulseCanada.2009. Chickpea. http://www.pulsecanada.com/what-are-pulses/chickpeas. Verified on November 20, 2009.

Ramanujam, S. and V.P. Gupta. 1974. Stability of yield and its components in Bengal gram and its bearing on plant type. Indian Journal of Genetics 34:757-763.

Rao, P.S. 1976. Nature of carbohydrates in pulses. Journal of Agriculture and Food Chemistry 24:958–961.

Redden, R.J. and J.D. Berger. 2007. History and origin of chickpea. In: S.S. Yadav, R. Redden, W. Chen and B. Sharma (eds.). Chickpea Breeding and Management. CAB International. pp. 1-13.

Redden, R.J., B.J. Furman, H.D. Upadhyaya, R.P.S. Pundir, C.L.L. Gowda, C.J. Coyne, and D. Enneking. 2007. Biodiversity management in chickpea. In S.S. Yadav, R. Redden, W. Chen and B. Sharma (eds.). Chickpea Breeding and Management.CAB International, Wallingford, UK. pp. 355-368.

Ribeiro, J.M.C.R. and I.M.P. Melo. 1990. Composition and nutritive value of chickpea. Options Mediterraneennes 9:107-111.

Roman, R.M., C.J. Wilcox and F.G. Martin. 2000. Estimates of repeatability and heritability of productive and reproductive traits in a herd of Jersey cattle. Genetics and Molecular Biology 23:113–119.

Roy, D. 2000. Plant Breeding-Analysis and exploitation of variation. Alpha Science International Ltd, Pangbourne, UK. pp. 163-198.

Rubio, J., F. Flores, M.T. Moreno, J.I. Cubero and J. Gil. 2004. Effects of the erects/bushy habit, single/double pod and late/early flowering genes on yield and seed size and their stability in chickpea. Field Crops Research 90:255-262.

Saini, H.S. and E.J. Knights 1984. Chemical Constitution of Starch and Oligosaccharide Components of Desi and Kabuli Chickpea (*Cicer arietinum*) Seed Types. Journal of Agriculture and Food Chemistry 32:940-944.

Salgado, P., J.P. Lalles, R. Toullec, M. Mourato, F. Cabraland, J.P.B. Freire. 2001. Nutrient digestibility of chickpea (*Cicer arietinum* L.) seeds and effects on the small intestine of weaned piglets. Animal Feed Science and Technology 91:197-212.

Salmeron, J., J.E. Manson, M.J. Stampfer, G.A. Colditz A.L. Wing W.C. Willett. 1997. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. The Journal of the American Medical Association 277: 472–477.

Sandhu, K.S. and S. Lim. 2008. Digestibility of legume starches as influenced by their physical and structural properties. Carbohydrate Polymers 71:245-252.

Sanchez-Mata, M. C., M. J. Penuela-Teruel, M. Camara-Hurtado, C. Diez-Marques and M. E. Torija-Isasa. 1998. Determination of mono-, di-, and oligosaccharides in legumes by high-performance liquid chromatography using an amino-bonded silica column. Journal of Agricultural and Food Chemistry 46:3648–3652.

Sasaki, T., T. Yasui, J. Matsuiki and T. Satake. 2002. Comparison of physical properties of wheat starch gels with different amylose content. Cereal Chemistry 79:861-866.

SAF (Saskatchewan Agriculture and Food), Agricultural Statistics- Specialty crops:acreage,yieldandproduction. Title. [Online]. (2007). Available: www.agr.gov.sk.ca [Last accessed: 24 March 2007].

Saskatchewan Pulse Growers, 2009. Overview of Pulses. http://www.saskpulse.com/producer/industry/index.php. Verified on October 8 2009.

Saura-Calixto, F., I. Goni, L. Bravo and E. Manas. 1993. Resistant starch in foods: Modified method for diatery fibre residues. Journal of Food Science 58:642-643.

Scheiner, S.M. and R.F. Lyman. 1989. The genetics of phenotypic plasticity I. Heritability. Journal of Evolutionary Biology 2:95-107.

Schroeder, H.E. 1982. Quantitative studies on the cotyledonary proteins in the genus *Pisum*. Journal of the Science of Food and Agriculture 33:623–633.

Segherloo, A.E., S.H. Sabaghpour, H. Dehghani, M. Kamrani. 2008. Non-parametric measures of phenotypic stability in chickpea genotypes (*Cicer arietinum* L.). Euphytica 162:221–229.

Sekhon, H.S. and G. Singh. 2007. Irrigation management in chickpea. In: Chickpea Breeding and Management. S.S Yadav, R. Redden, W. Chen, B. Sharma (eds). CABI International, Wallingford, pp. 246-267.

Serraj, R., L. Krishnamurthy and H.D. Upadhyaya. 2004. Screening chickpea mini core germplasm collection for tolerance to soil salinity. International Chickpea and Pigeonpea Newsletter. 11:29-32.

Serret D.M., S.M. Udupa and F. Weigand 1997. Assessment of genetic diversity of cultivated chickpea using microsatellitederived RFLP markers: implications for origin. Plant Breeding 116: 573–578.

Shannon, C.E., and W.Weaver. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, USA.

Sharma, V., K.D. Rausch, J.V. Graeber, S.J. Schmidt, P. Buriak, M.E. Tumbleson, and V. Singh. 2010. Effect of resistant starch on hydrolysis and fermentation of corn starch for ethanol. Applied Biochemistry and Biotechnology 160:800-811.

Shi, Y.C. and P.A. Seib. 1992. The structure of four waxy starches related to gelatinization and retrogradation. Carbohydrate Research 227:131-145.

Shibanuma, K., Y. Takeda, S. Hizukuri and S. Shibata. 1994. Molecular structures of some wheat starches. Carbohydrate Polymer 25:111-116.

Shu, X., L. Jia, H. Ye, C. Li and D. Wu. 2009. Slow digestion properties of rice different in resistant starch. Journal of Agriculture and Food Chemistry 57:7552-7559.

Shu, X., L. Jia, J. Gao, Y. Song, H. Zhao, Y. Nakamura and D.Wu. 2007. The Influences of Chain Length of Amylopectin on Resistant Starch in Rice (*Oryza sativa* L.). Starch/Starke, 59: 504-509.

Sika, M., A. Terrab, P.B. Swan and P.V.J. Hegarty 1995. Comparison of selected Moroccan cereals and legumes: Comparison with the FAO table for use in Africa. Journal of Food Composition and Analysis 8:62-70.

Silvester, K.R., H.N. Englyst and J.H. Cummings. 1995. Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermented of ileal effluent. American Journal of Clinical Nutrition 62:403-411.

Singh, N., K. S. Sandhu and M. Kaur. 2004. Characterization of starches separated from Indian chickpea (*Cicer arietinum* L.) cultivars. Journal of Food Engineering 63:441-449.

Singh, K.P., V.P. Singh and P.K. Sareen. 1995. Stability of yield and its components in chickpea (*Cicer arietinum* L.). International Journal of Tropical Agriculture 13:191-197.

Singh, O. and S. Kumar. 1994. Phenotypic stability of yield and related characters in desi gram (*Cicer arietinum* L.). Indian Journal of Agricultural Science 64:815-820.

Singh, K.B., G. Bejiga and R.S. Malhotra. 1993. Genotype–environment interactions for protein content in chickpea. Journal of the Science of Food and Agriculture 63:87–90.

Singh, P.K., N.B. Singh and R.C. Bharti. 1993. Divergence and stability analysis in chickpea. Crop Improvement 20:59-62.

Singh, G., H.S. Brar and M.M. Verma 1991. Genotype environment interaction and phenotypic stability in chickpea. Crop Improvement 18:135-140.

Singh, V. and F. Singh. 1991. Stability of yield and yield component characters in chickpea. Indian Journal of Genetics and Plant Breeding 51:183-189.

Singh, U. 1985. Nutritional quality of chickpea (*Cicer arietinum* L.): current status and future research needs. Plant Foods for Human Nutrition 35:339-351.

Singh, U., J. Kumar and C.L.L. Gowda. 1983. The protein content of chickpea (*Cicer arietinum* L.) grown at different locations. Plant Foods for Human Nutrition 32:179–184.

Singh, S., N. Nath and H.P. Nath. 1956. Iodine binding capacity of some native starches of Indian food seeds. Biochemistry Journal 63:718–722.

Skrabanja, V., H.G.M. Liljeberg, C.L. Hedley, I. Kreft and M.E Bjorck. 1999. Influence of genotype and processing on the invitro rate of starch hydrolysis and resistant starch formation in peas (*Pisum sativum* L.). Journal of Agriculture and Food Chemistry 47:2033-2039.

Slinkard, A.E., M.B. Soth and A. Vandenberg. 2000. Breeding for yield: the direct approach. In: Knight, R. (ed.) Linking Research and Marketing Opportunities for Pulses in the 21st Century. Kluwer Academic, Dordrecht, The Netherlands, pp. 1183-1190.

Sood, B.C., S.K. Bhambota and S.L. Gartan. 2001. Studies on genotype x environment interaction and stability in chickpea. Indian Journal of Pulses Research 14:31-33.

Soral-Śmietana, M., A. Swigona, R. Amarowicz and L. Sijtsma. 1998. The solubility of trypsin pea protein hydrolysates. Nahrung 42:217–218.

Soral-Śmietana, M. and Z. Dziuba. 1995. Changes in fraction structure of faba bean starch. *In*: AEP (ed.) Proceedings of the second AEP conference. Improving Production and Utilisation of Grain Legumes. Copenhagen. AEP, Paris, pp. 402-403.

Statistical Analysis Systems (SAS) Institute, 1989. SAS/STAT user guide. Version 6, 4th ed. SAS Inst., Inc, Cary, N.C.

Stevnebo, A., S. Sahlstrom and B. Svihus. 2006. Starch structure and degree of hydrolysis of small and large starch granules from barley varieties with varying amylose content. Animal Feed Science and Technology 130:23-38.

Sun, W. Q. and A. C. Leopold. 1995. The Maillard reaction and oxidative stress during aging of soybean seeds. Physiologia Plantarum 94:94-104.

Svihus, B., A.K. Uhlen and O.M. Harstad. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Animal Feed Science and Technology 122:303-320.

Takeda, Y., S. Hizukuri and B.O Juliano. 1987. Structures of rice amylopectin with low and high affinities for iodine. Carbohydrate Research 168: 79-88.

Takeda, Y., S. Hizukuri and B.O. Juliano. 1986. Purification and structure of amylose from rice starch. Carbohydrate Research 148:299-308.

Takeda, C., Y. Takeda and S. Hizukuri. 1983. Physicochemical studies of lily starch. Cereal Chemistry 60:212-216.

Tatsumi, H., H. Katano and T. Ikeda. 2007. Kinetic analysis of glucoamylase-catalyzed hydrolysis of starch granules from various botanical sources. Bioscience Biotechnology and Biochemistry 71:946-950.

Tester, R.F., J. Karkalas and X. Qi. 2004. Starch structure and digestibility enzyme-substrate relationship. World's Poultry Science Journal 60:186-195.

Tetlow, I.J. 2006. Understanding storage starch biosynthesis in plants: a means to quality improvement. Canadian Journal of Botany 84:1167-1185.

Tetlow, I.J., K.G. Beisel, S. Cameron, A. Makhmoudova, F. Liu, N.S. Bresolin, R. Wait, M.K. Morell and M.J. Emes. 2008. Analysis of protein complexes in wheat amyloplasts reveals functional interactions among starch biosynthetic enzymes. Plant Physiology 146:1878-1891.

Tetlow, I.J., R. Wait, Z. Lu, R. Akkasaenga, C. G. Browsher, S. Esposito, B. Koshar-Hashemi, M. K. Morell and M. J. Emes. 2004. Protein phosphorylation in amyloplasts regulates starch branching enzyme activity and protein-protein interactions. Plant Cell 16:694-708.

Thacker, P.A., S. Qiao and V.J. Racz. 2002. A comparison of the nutrient digestibility of desi and kabuli chickpeas fed to swine. Journal of the Science of Food and Agriculture 82:1312-1318.

Tharanathan, R.N. and S. Mahadevamma 2003. Grain legumes- a boon to human nutrition. Trends in Food Science and Technology 14:507-518.

Thorne, M.J., L.U. Thompson and D.J.A. Jenkins 1983. Factors affecting starch digestibility and the glycemic response with special reference to legumes. American Journal of Clinical Nutrition 38:481-488.

Tomer, G.S., L. Singh, D. Sharma and A.D. Deodhar. 1973. Phenotypic stability of yield and some seed characteristics in Bengal gram (*Cicer arietinum* L.) varieties. Jawaharlal Nehru Krishi Vishwa Vidyalaya Journal 7:35-39.

Tomomatsu, H. 1994. Health effects of oligosaccharides. Food Technology 48:61-65.

Topping, D. L. and P. M. Clifton. 2001. Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides. Physiological Reviews 81:1031-1064.

Ultrilla-Coello, R.G., P. Osorio-Diaz and L.A. Bello-Perez. 2007. Alternative use of chicpea flour in breadmaking: chemical composition and starch digestibility of bread. Food Science and Technology International 13:323-327.

Upadhyaya, D.H., B.J. Furman, S.L. Dwivedi, S.M. Udupa, C.L.L. Gowda, M. Baum., J.H. Crouch, H.K. Buhariwalla and S. Singh 2006a. Development of a composite collection for mining germplasm possessing allelic variation for beneficial traits in chickpea. Plant Genetic Resources 4:13-19.

Upahdyaya, H.D., S. Kumar, C. Gowda and S. Singh 2006b. Two major genes for seed size in chickpea (*Cicer arietinum* L.). Euphytica 147:311-315.

Upadhyaya, H.D. 2003. Geographical patterns of variation for morphological and agronomic characteristics in the chickpea germplasm collection. Euphytica 132:343-352.

Upadhyaya, H.D. and R. Ortiz. 2001. A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. Theoretical and Applied Genetics 102:1292-1298.

Upadhyaya, H.D., P.J. bramel and S. Singh. 2001. Development of chickpea core subsetusing geographical distribution and quantitative traits. Crop science 41:206-210.

van der Maesen, L.J.G., N. Maxted, F. Javadi, S. Coles and A.M.R. Davies. 2007. Taxanomy of the genus *Cicer* revisited. In: S.S. Yadav, R. Redden, W. Chen, B. Sharma (eds.). Chickpea Breeding and Management, CABI International, Wallingford, pp. 14-46.

van der Maesen, L.J.G. 1987. *Cicer* L. Origin, history and taxonomy of chickpea. p.11-34. In: M.C. Saxena and K.B. Singh (ed.), The Chickpea. C.A.B. International Cambrian News Ltd, Aberystwyth, UK.

van der Maesen, L.J.G.1972. *Cicer* L., a monograph of the genus, with special reference to the chickpea (*Cicer arietinum* L.), its ecology and cultivation. Mededelingen Landbouwhogeschool (Communications Agricultural University) Wageningen. The Netherlands 72–10.

Vandenberg, A. T. Warkentin and A.Slinkard. 2004. CDC Desiray desi chickpea. Canadian Journal of Plant Sciences 84: 795-796.

Vasanthan, T. 2001. Enzymatic quantification of total starch in plant products. Current Protocols in Food Analytical Chemistry E2.2.1-E2.2.9.

Vesterinen, E., P. Myllarinen, P. Forssell, E. Soderling and K. Auto. 2002. Structural properties in relation to oral enzymatic digestibility of starch gels based on pure starch components and high amylose content. Food Hydrocolloids 16:161-167.

Villareal, C.P., N.M. De La Cruz and B.O. Juliano. 1994. Rice amylose analysis by near infrared transmittance spectroscopy. Cereal Chemistry 71: 292-296.

Vishnu-Mittre and R. Savithri 1982. Food economy of the Harappans. In: Possehl G.L. (ed), Harrapan Civilization. A contemporary Perspective, Oxford and IBH, New Delhi, pp. 205–221.

Viveros, A., A., Brenes, R. Elices, I. Arija and R. Canales. 2001. Nutritional value of raw and autoclaved kabuli and desi chickpeas (*Cicer arietinum* L.) for growing chickens. British Poultry Science 42:242-251.

Wang, T. L., C. Domoney, C. L. Hedley, R.Casey and M. A. Grusak. 2003. Can We Improve the Nutritional Quality of Legume Seeds? Plant Physiology 131:886-891.

Wang, T.L., T.Y. Bogracheva and C. Hedley. 1998. Starch: as simple as A, B, C?. Journal of Experimental Botany 49 481-502.

Ward, J. 1963. Hierarchical grouping to optimize an objective function. Journal of American Statistical Association 38:236-244.

Welsh, L.A and A.B. Blakeney. 1992. Choosing an amylose standard for cereal starch analysis. Proc. 42nd Aust. Cereal Chem. Conf. (V.J. Humphrey-Taylor, ed). Royal Australian Chemical Institute, Parkville, Vic. pp. 347-350.

Wilcoxon, F. 1945. Individual comparisons by ranking methods. Biometric Bulletin 1:80-83.

Wood, J.A., E.J. Knights and S. Harden. 2008. Milling performance in desi-type chickpea (*Cicer arientinum* L.): effects of genotype, environment and seed size. Journal of the Science of Food and Agriculture 88:108–115.

Wood, J.A. and M.A. Grusak. 2007. Nutritional value of chickpea. In: S.S. Yadav, R., Redden, W. Chen and B. Sharma (eds).Chickpea Breeding and Management.CAB International, Wallingford, UK. pp. 101-142.

XinLing, Y., D. XiangZhen, C. YanYun, W. LinKe, W. Bing, W. YanFan and G. AiGuang. 2005. Correlativity between apparent starch contents and amylose contents in potato. Acta Botanica Boreali–Occidentalia Sin 25:953–957.

Yadav, S.S., N. Longnecker, F. Dusunceli, G. Bejiga, M. Yadav, A.H. Rizvi, M. Manohar, A.A. Reddy, Z. Xaxiao, and W. Chen. 2007. Uses, Consumption and Utilization. In: S.S. Yadav, R. Redden, W. Chen, and B. Sharma (eds). Chickpea Breeding and Management.CAB International, Wallingford, UK. pp. 72-100.

Yadava, H.S., K.V.V. Prasad and S.C. Agrawal. 2000. Stability in resistance against wilt in chickpea (*Cicer arietinum* L.). Indian Journal of Agricultural Sciences 70:345-348.

Yadavendra, J.P. and S.K. Dixit. 1987. Stability for seed protein in chickpea. Indian Journal of Agricultural Sciences 57:876-879.

Yamada, Y. 1962. Genotype by environment interaction and genetic correlation of the same trait under different environments. The Japanese Journal of Genetics 37:498-509.

Yan, W. and N.A. Tinker. 2005. An integrated biplot analysis system for displaying, interpreting, and exploring genotype × environment interaction. Crop Science 45:1004–1016.

Yan, W. 2002. Singular-value partitioning in biplot analysis of multi environment trial data. Agronomy Journal 94:990–996.

Yan, W. 2001. GGE biplots – Windows application for graphical analysis of multi-environmental trial data and other type of two-way data. Agronomy Journal 93:1111–1118.

Yao, Y., M. J. Guiltinan and D. B. Thompson. 2005. High-performance size-exclusion chromatography (HPSEC) and fluorophore-assisted carbohydrate electrophoresis (FACE) to describe the chain-length distribution of debranched starch. Carbohydrate Research 340: 701–710.

Yasui, T., T. Sasaki and J. Matsuki. 2005. Variation in the chain-length distribution profiles of endosperm starch from *Triticum* and *Aegilops* species. Starch/Starke 57:521-530.

Yu, X., C. Houtman and R.H. Atalla. 1996. The complex of amylose with iodine. Carbohydrate Research 292:129-141.

Zeeman, S.C., J. Kossman and A.M. Smith. 2010. Starch: Its metabolism, evolution, and biotechnological modification in plants. Annual Review of Plant Biology 61:209-234.

Zhao, X.C., and P.J. Sharp. 1996. An improved 1-D SDS-PAGE method for the identification of three bread wheat '*waxy*' proteins. Journal of Cereal Science 23:191–193.

Zhang, G., M. Sofyan, and B.R. Hamaker. 2008. Slowly digestible state of starch: Mechanism of slow digestion property of gelatinized maize starch. Journal of Agriculture and Food Chemistry 56: 4695-4702.

Zhu, T., D. S. Jackson, R.L. Wehling and B. Geera. 2008. Comparison of Amylose Determination Methods and the Development of a Dual Wavelength Iodine Binding Technique. Cereal Chemistry 85:51–58.

Zohary, D. 1989. Pulse Domestication and Cereal Domestication: How Different are They?. Economic Botany 43:31-34.