

PHASIC CHANGES IN AbA LEVELS
IN WHEAT (Triticum aestivum L.)
DURING DROUGHT STRESS

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ABSTRACT

This study was designed to determine how endogenous AbA levels in wheat plants changed when drought stress was imposed at various stages of its development and to select a stage of development at which changes in AbA levels were most sensitive to drought stress. Sensitivity in this sense was synonymous with the production of large amounts of AbA in response to drought stress.

The variety used in this study was Pitic 62 and the plants were grown under controlled environmental conditions. At each of the five stages of development studied, tillering, stem elongation, booting, inflorescence emergence and milk development stages, stress was imposed on the plants up to five days of stress. Control plants were sampled on days corresponding to alternate days of stress. Parameters of plant water status studied were total leaf water potential, leaf osmotic potential, leaf pressure potential and relative water content. Changes in these parameters were related to changes in AbA levels, stomatal resistance and dry matter accumulation.

It was found that there were clear differences in drought-induced increases in AbA levels between the stages of development. There was also a tendency to produce greater AbA levels in response to stress at later stages of development. The implications of these findings have been discussed. Relative increases in AbA levels were found not to differ greatly from stage to stage suggesting the possibility that it may be an inherent capability of the cultivar or crop.

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1. INTRODUCTION

The development of crop plants is known to be affected by moisture deficits (Hsiao, 1973). Although a lot of work has been done on the effects of water stress on crop yield, only empirical studies have been made of these stress effects on the physiology of plants at different stages of their development. It is now widely believed that abscisic acid (AbA) is the key hormone which directly, or indirectly, controls the physiological processes in plants affected by water stress (Davies and Mansfield, 1983). This belief compounded with the fact that there is genotypic variation in AbA accumulation in cereals (Quarrie, 1981; Henson, 1980) and that this capacity is a highly heritable character (Austin, Henson and Quarrie, 1982) has lead to the use of AbA accumulation as a screening tool for drought resistance (Henson, 1981; Quarrie, 1981).

In determining genotypic variation in AbA levels during drought stress, arbitrary stages of growth were used in previous studies (Larque-Saavedra and Wain, 1976). AbA differences were studied in detached and wilted leaves of maize and sorghum varieties at the young seedling stage when the second leaf with ligules was formed. Quarrie and Jones (1979) studied genotypic variation in AbA in intact young leaves of wheat seedlings drought stressed when 5 or 6 leaves had emerged. Quarrie (1980) used a similar procedure in

studying AbA accumulation during drought stress in 26 wheat varieties. It is not known from this work whether genotypic differences in the sensitivity of wheat to AbA would be maintained at later stages of development. Ilahi and Dorffling (1982) studied genotypic differences in AbA levels in four maize varieties stressed at monthly periods up to three months and found genotypic differences in AbA levels in the one-month-old plants but less pronounced differences in the two-month-old plants and no observable differences in the three-month-old plants. Phasic changes in AbA levels, however, were clearly observable in all varieties.

The present study was designed to determine the nature of association between developmental stages during drought stress and variations in AbA levels for a single wheat cultivar and to determine the stage best suited for drought studies in relation to AbA changes.

2. LITERATURE REVIEW

2.1 Occurrence of AbA

Since being recognized as an important endogenous regulator in its own right (Kefford, 1963), AbA has now been identified in a great variety of species and appears to occur universally among vascular plants. With the increasing use of sophisticated analytical techniques and assay methods, AbA has now been detected in almost every part of the higher plant and in highest concentrations in leaves, buds, fruits and seeds.

AbA is generally an inhibitor of plant growth and development and is particularly associated with bud dormancy, abscission, seed dormancy, root growth and geotropism, inhibition of flowering and senescence (Addicott and Carns, 1983). In low concentrations, however, AbA shows stimulatory effects in parthenocarpy, rooting of cuttings, growth of root tips and callus growth. AbA's role in inducing stomatal closure is well established and its accumulation during water stress has been well documented.

2.2 Evidence for the association of AbA with water stress.

The first evidence relating water stress to AbA production was presented by Wright (1969). In his study, excised leaves from wheat seedlings were wilted and changes in the amount of AbA (then known as inhibitor- β) were noted.

Wright found that the AbA content was proportional to wilting. Within four hours, a forty-fold increase in AbA was detected. Mizrahi et al. (1970) observed that when osmotic stress was applied to tobacco plants, the AbA content in the leaves increased and remained high even upon recovery of turgor by the leaf.

There have since been many reports supporting the findings of Wright (1969) and Mizrahi et al. (1970). Most (1971) found that AbA accumulated in relatively large amounts in the leaves of sugarcane plants subjected to drought stress. In addition to AbA, phaseic acid, a metabolite of AbA was also detected indicating its possible role during water stress. In spinach plants, Zeevaart (1971) found a ten-fold increase in AbA content in wilted plants over that of turgid plants. Hiron and Wright (1973) found that inducing a leaf water deficit by warm air treatment caused an increase in AbA levels in the leaves of dwarf bean seedlings. They also found that AbA levels remained high for a period after leaves had regained full turgor. The rapidity of increase in AbA levels during water stress was illustrated by Loveys and Kriedmann (1973) when they showed that AbA levels in vine leaves doubled within fifteen minutes of their excision. A forty-four-fold increase was observed in these leaves after six days of stress. AbA levels rapidly declined following rewatering of plants which was in contrast to the findings of Mizrahi et al. (1970), and Hiron and Wright (1973). Kannangara et al. (1982a) found a negative relationship between leaf water potential and the amount of AbA produced in the leaves of field grown sorghum

plants during all but a six-hour period during the day.

Zabadal (1974) proposed the concept of a threshold leaf water potential at which AbA synthesis is triggered off under water stressed conditions. He found the critical range to be between -1.0 and -1.2 MPa in Ambrosia artemisifolia and Ambrosia trifida while Beardsell and Cohen (1975) reported the range to be between -0.8 and -1.0 MPa in excised leaves of maize and sorghum. Walton, Galson and Harrison (1977) observed sharp increases in AbA levels when leaf water potential in bean seedlings was between -0.7 and -0.9 MPa. In excised wheat leaves, Wright (1977) found the threshold value to be at -0.9 MPa. However, Sivakumaran and Hall (1978), could not find any evidence of a threshold potential in osmotically stressed plants of Euphorbia lathyrus.

Pierce and Raschke (1980, 1981) presented evidence that in detached leaves, the accumulation of AbA during water stress is a function of reduced turgor potential rather than leaf water potential per se. Decreasing water potential had little effect on AbA levels in leaves of Phaseolus vulgaris, Xanthium strumarium and Gossypium hirsutum at high turgor. Sensitivity of the production of AbA to changes in leaf water potential increased as turgor approached zero and recovery of turgor caused acceleration of AbA by metabolism. This finding was supported by Henson (1982) who showed that bulk leaf AbA content of stressed rice plants increased linearly as turgor potential declined. It therefore appears that turgor is the controlling component of leaf water potential in stimulating

AbA production during drought stress. However a correlation could not be established between the diurnal changes in AbA and turgor potentials under field conditions. Diurnal studies in millet (Henson et al, 1982a) showed that two maxima of AbA content were achieved during the day. Neither maxima corresponded with the lowest leaf water potentials during the day. Henson et al. (1982a) showed that the AbA content during the day was not always accounted for by changes in turgor potential or leaf water potential. More recently, Loveys and During (1984) studied diurnal changes in water relations in field grown vine cultivars and observed that AbA did not consistently accumulate in attached leaves in response to zero turgor, even if this condition was maintained for the greater part of the day. This discrepancy between AbA content and turgor potential in field grown plants may be explained by the findings of Setter et al. (1980), who showed that AbA can be translocated out of the leaves in the same manner as photosynthetic assimilates. Furthermore, Hoad (1975) showed that AbA may be translocated to the leaves through the xylem. The failure of intact leaves to accumulate AbA may thus be due to rapid phloem loading while the accumulation of AbA at higher turgor as observed by Henson et al. (1982a) and Loveys and During (1984) may be due to translocation into the leaves via the xylem.

2.3 Involvement of AbA in stomatal responses due to drought stress.

Early evidence for the existence of a chemical "distress signal" was provided by Glover (1959) who showed that despite recovery of leaf turgor in plants which had experienced a period of wilting, the reopening of stomata was delayed. The association of AbA with stomatal movements was first implied by Mittelheuser and Van Steveninck (1969) who showed that application of AbA via the transpiration stream induced stomatal closure and inhibited transpiration in excised first leaves of wheat and barley. Tal and Imber (1970) observed that in the wilty tomato mutant, flacca, water stress kept stomata open while AbA levels were low. However, exogenously applied AbA closed the stomata. Jones and Mansfield (1970) applied small doses of AbA to the leaf surfaces of Xanthium pennsylvanicum and found stomatal closure was induced.

Direct evidence for the involvement of endogenous AbA in controlling stomatal closure was provided by Wright (1972), who showed a strong correlation between leaf resistance and the endogenous level of AbA throughout the wilting and recovery cycle of Brussel sprouts plants. Hiron and Wright (1973) further showed that when leaves of dwarf bean were subjected to water stress, there was an increase in endogenous levels in the leaves, accompanied by an increase in stomatal resistance. In a study of the sequential responses of rice to water stress, Henson (1982) showed that the first response in

the leaves was a rise in AbA content followed by closure of stomata and then the initiation of leaf rolling.

Allaway and Mansfield (1970) suggested that AbA remains at enhanced levels after the removal of stress and inhibits stomatal opening. Several studies on the after-stress effects on AbA levels have shown that levels decline slowly but still inhibit stomatal opening. Generally an inverse relationship between AbA levels and stomatal opening is observed (Beardsell and Cohen, 1975). Dorffling and coworkers (1977) observed a delay in the opening reaction of the stomata relative to recovery of turgor in the leaves of Pisum sativum, Helianthus annuus and Vicia faba. The duration of this after-effect of stress on stomatal opening was directly correlated with the AbA content in the leaves.

2.4 Inconsistencies in AbA regulation of stomatal conductance.

Although there is sufficient evidence to establish the fact that AbA can induce stomatal closure, there have been several reports that cast doubts on the existence of a straight-forward relationship between leaf AbA levels and stomatal conductance. Beardsell and Cohen (1975) found in the leaves of maize plants, a rise in stomatal resistance preceding any marked increase in AbA content of the leaves during the early stage of stress. Walton et al. (1977) failed to observe any AbA increases prior to initiation of stomatal closure in the leaves of stressed Phaseolus vulgaris plants.

Ackerson (1980) working on cotton plants found that the amount of AbA required to initiate stomatal closure in leaves during stress was not related to the amounts accumulated during wilting. Newville and Ferrel (1980) too, found no correlation between AbA levels and stomatal closure in the leaves of stressed Douglas-fir seedlings. Henson (1981a) further demonstrated that while stomatal closure was virtually complete within 20 minutes from shoot excision of well watered pearl millet, an increase in bulk AbA content was not detected until 25 to 30 minutes.

These observations have prompted a few hypotheses to explain why stomatal behaviour may be unrelated to leaf AbA concentrations. Several measurements of the distribution of AbA within the leaf cells provide an explanation for the observation that water-stress induced stomatal closure can precede build-up of AbA in the plant. Loveys (1977) found that AbA in the leaves of well watered plants was nearly all located in the mesophyll chloroplasts. It, therefore, seems that closure of stomata during the early stages of this water stress could be due to the release of this stored AbA and not dependent on the synthesis of new AbA. The concept of compartmentation was introduced to account for the fact that AbA is found in large quantities in the mesophyll chloroplasts. Some of these compartments in which AbA is found may be connected to guard cells while others may not (Raschke et al. 1976 and Raschke and Zeevaart, 1976). Studies have shown that the amount of AbA required to induce stomatal

closure was about an order or two less than the amount of AbA stored in the chloroplasts of well watered plants (Raschke, 1982). Raschke (1975a) found that the amount of AbA, fed to a leaf of Xanthium strumarium through the transpiration stream, required to reduce stomatal conductance by 5% of its initial value, was less than 2% of the original AbA content of the leaves. Studies by Weyers and Hillman (1979) in which radioactive AbA was applied to leaves of Commelina communis, showed that stomatal closure was correlated with increases in epidermal AbA contents ranging between 0.8 and 2.7 fmolmm⁻². The amount of AbA in a whole leaf was estimated to be 5.6 pmolmm⁻². Pierce (1981) found that AbA content in epidermis of Commelina communis rose by less than 1 fmolmm⁻² when stomata closed. These observations suggest that the release and redistribution of AbA already present in the mesophyll chloroplasts is sufficient to bring about the closure of stomata in the early stages of water stress, prior to any new synthesis of AbA.

There have been some reports on the penetrability of plastid envelopes to AbA (Wellburn and Hampp, 1976; Gimmler, Hartung and Heilmann, 1979) and this raises the possibility that changes in membrane permeability could occur in response to drought stress. If an increase in membrane permeability to AbA occurred early at the onset of water stress, then stomatal closure would occur without the necessity for de novo synthesis of AbA. Synthesis of new AbA itself would be promoted by this release into the 'compartments' which are connected to the guard cells (Davis, Mansfield and Wellburn,

1979).

This raises the question of why AbA needs to be synthesized de novo in extravagant quantities in response to drought stress when only a fraction of this amount is enough to induce stomatal closure. Although de novo synthesis upon turgor loss (Pierce and Raschke, 1980) will not serve as a stress signal, since stomata are already closed, it may serve as a mechanism that prepares the plant for further episodes of stress (Raschke, 1982). The slow metabolic removal of AbA would ensure a gradual return to normalcy when stress is removed. This also explains the after-stress effects of AbA accumulation in some plants (Beardsell and Cohen, 1975; Dorffling et al., 1977; Walton, 1980).

Levitt (1980) proposed that the closing of stomata prior to any detectable increase in AbA is not necessarily due to a redistribution of available AbA. Instead the closure is simply a hydropassive action due to passive loss of water from the guard cells. However, AbA has a longer lasting effect on stomatal closure than hydropassive action and, therefore, remains even after rehydration.

Several studies have been done to detect if there are other substances released in the leaves which induce stomatal closure prior to AbA synthesis. Ogunkanmi, Wellburn and Mansfield (1974), identified all-trans-farnesol in stressed sorghum leaves which, when reapplied to the leaves of non-stressed sorghum plants, closed stomata in the same manner as AbA. Mansfield, Wellburn and Moreira (1978), however, suggest

that farnesol acts to alter the permeability of chloroplasts to AbA, allowing AbA to enter the cytoplasm and thence to the guard cells. Harrison and Walton (1975) found that metabolites of AbA, phaseic acid (PA) and dihydrophaseic acid (DPA) increased following water stress. However PA is only half as effective as AbA while DPA has no effect at all on stomatal closure (Sharkey and Raschke, 1980). Xanthoxin has been found to be a naturally occurring antitranspirant in leaves but its levels do not increase when stress is imposed (Zeevaart, 1974).

Although all these compounds are known to induce stomatal closure to varying degrees, no conclusive evidence has been presented to implicate them in stomatal closure prior to detectable increases in AbA levels.

Raschke (1982) sums up these hypotheses on AbA inconsistencies in stomatal regulation by the following:

"that the exact synchrony of stomatal movement with changes in AbA levels in leaves and the occurrence of stress can a priori not be expected."

In contrast to the notion that elevated levels of AbA remain for a period after the removal of stress, several studies on the after-effects of stress have shown that AbA may be absent during recovery from stress. Loveys and Kriedmann (1973) showed that after a forty-four-fold increase in AbA during prolonged stress in vine leaves, it rapidly declined following rewatering. Bengtson, Falk and Larsson (1977), found no direct relationship between the after-effect and the AbA

content of the leaf. They showed that in young stressed wheat plants during recovery, the AbA content reattained the pre-stressed level within three hours while transpiration achieved pre-stressed levels at least ten hours later. Henson (1981b) likewise showed that in intact stressed plants of pearl millet, AbA decreased to control levels 8 hours after rewatering while conductance recovered to control levels 48 hours after rewatering. These observations may be explained in terms of the metabolism of AbA as it returns to pre-stressed levels (Walton, 1980). The metabolic PA inhibits stomatal opening even as AbA content returns to pre-stress levels. The concept of compartmentation can be implied here again. Since only very small amounts are needed to keep stomata closed, the rest of the AbA may return to pre-stress levels by metabolism.

Field grown plants experience repeated episodes of stress and several groups have shown that stomatal response is insensitive to changes in AbA levels. This insensitivity may be due to adaptation in the guard cells as a result of repeated encounters with stress (Henson, 1983). This probably explains why field grown vine (Loveys and During, 1984) or rice (Henson et al., 1982b) plants may keep their stomata open even in the presence of high AbA levels especially in the later part of the day. Therefore, environmental variables such as temperature, atmospheric vapor pressure deficit, irradiance and CO₂ levels become more accountable for stomatal regulation than changes in AbA levels (Henson et al., 1982b; Loveys and During, 1984; Raschke, 1975b).

2.5 Synthesis and mode of action of AbA during drought stress

Loveys (1977), Dorffling et al. (1979) and Pierce (1981) have all shown that AbA is not synthesized by the stomatal complex but is transported there from the mesophyll. Since chloroplast activities are severely inhibited by water stress (Boyer, 1976), it makes them an appropriate location for the trigger that responds to changes in turgor or water potential during water stress. Although guard cells contain chloroplasts too, they may not be a suitable location for sensing stress because they experience large diurnal changes in turgor and solute potential (Davies and Mansfield, 1983). The hypothesis that AbA is largely synthesized in the plastids has had considerable support (Milborrow, 1974; Loveys, 1977; Heilmann et al., 1980). Milborrow (1974) reported that isolated chloroplasts are able to incorporate mevalonic acid (MVA), a biosynthetic precursor of AbA, into AbA. Loveys (1977) showed that chloroplasts from unstressed spinach contain nearly all of the AbA in the leaf although in stressed leaves most of the AbA appears to be extrachloroplastic. Heilmann et al. (1980) supported Lovey's findings when they reported that 75-80% of the AbA in unstressed spinach leaves is in the chloroplasts.

Mansfield et al. (1978) and Milborrow (1979) have suggested that water stress causes an increase in the permeability of the chloroplast membrane to AbA, which causes AbA to leak from the chloroplasts into the cytoplasm.

Mansfield et al. (1978) have suggested that farnesol, formed in increasing quantities as a result of water stress, might increase the chloroplast membrane permeability to AbA. Since AbA during water stress is released rapidly from the chloroplasts, biosynthesis of AbA would commence as the concentration within the chloroplasts falls. The reduction in chloroplast AbA concentration induces rapid de novo synthesis (Milborrow, 1979). The studies of Pierce and Raschke (1981), Zeevaart (1980) and Murphy (1984), all support the hypothesis that enhanced de novo synthesis rather than reduced degradation is the main process by which AbA levels are elevated following water stress. Upon rewatering and restoration of leaf turgor, the plastid membranes become impermeable to AbA again, and the biosynthesis of AbA would become inhibited by the restoration of the original concentration.

The skepticism of Walton (1980) that AbA may not necessarily be synthesized in the chloroplast lead Hartung et al. (1981) to demonstrate that AbA is synthesized in the cytoplasm and not in the chloroplasts. They found that labeled MVA was easily taken up by intact spinach chloroplasts but not converted to AbA. Similar observations were obtained from non-aqueously isolated chloroplasts. They rejected Milborrow's (1974) finding that isolated chloroplasts could synthesis AbA on the basis that the chloroplasts were contaminated with cytoplasm. Earlier, Heilmann et al. (1980) found that pH gradients between the organelles and cytoplasm could determine the release and movement of AbA. This lead Hartung et al.

(1981) to propose another hypothesis that AbA is synthesized in the cytoplasm and is distributed according to the pH gradient between the cytoplasm and the chloroplast stroma. The undissociated AbA is able to penetrate the chloroplast membranes and is trapped as anions in the alkaline stroma. Osmotic stress causes acidification of the stroma. This then decreases stroma pH which promotes the increase of AbA molecules into the undissociated state which are then able to penetrate the chloroplast membranes and be released to the cytoplasm. In addition to this rapid distribution of AbA, slow cytoplasmic synthesis of AbA takes place. Since stress-induced AbA release from the chloroplasts is more rapid than stress-induced closure of stomata, it explains the observations that stomatal closure occurs much earlier than stress-induced AbA biosynthesis.

Using inhibitors of protein synthesis on cytosol-ribosomes and plastid-ribosomes of barley leaves, Quarrie and Lister (1984a) showed that enzymes required for AbA production are synthesized in the cytosol and not in the plastid, indicating that enzymes for AbA synthesis are encoded in the nuclear DNA and not in the plastid DNA. Quarrie and Lister, later, (1984b) also showed that in white mutant leaves of albostrians, in which plastid ribosomes are completely absent, AbA accumulation during dehydration was severely restricted implying that plastid ribosomes contribute some factor that is necessary for AbA synthesis. Since cell growth and wall synthesis are the most sensitive processes to water stress

(Hsiao, 1973), the proximity of cell plasmalemma and/or cell wall to the transpiration stream puts them under strain before cell organelles during water stress. Using these concepts, Quarrie and Lister (1984a) developed a new hypothesis of AbA synthesis which they called the "cytosol enzyme and AbA synthesis model". The factor contributed by the plastid is a regulator or derepressor which may be a polypeptide or several polypeptides. This derepressor migrates across the plastid envelope to be incorporated into the plasmalemma and/or cell wall during cell development. In response to water stress, conformational changes at these sites in the plasmalemma and/or cell wall would activate the derepressor to switch on AbA synthesis in the cytosol. Upon rehydration, the derepressor would switch off AbA synthesis. This model clearly fits the finding of Hartung et al. (1981).

AbA synthesized in the mesophyll travels to the guard cells (Loveys, 1977; Pierce, 1981) where it inhibits potassium uptake into the guard cells (Weyers and Hillman, 1980), inhibits proton (H^+) release (Raschke, 1977) and promotes the release of malate (Van Kirk and Raschke, 1978). Epidermal cells act as sinks for potassium ions and malate excluded from the guard cells (Itai and Meidner, 1978). Inhibition of K^+ uptake and H^+ release and leakage of malate reduces the solute strength in the guard cells, thereby closing the stomata (Raschke, 1977).

When water stress is removed and rehydration occurs, the AbA accumulated in the leaves begins to metabolize mainly through oxidation to PA and DPA. AbA may also be conjugated to

AbA-glucose ester or other polar conjugated derivatives (Walton, 1980). Murphy (1984), however, showed that in wheat leaves, the predominant pathway of AbA metabolism is oxidation to PA and DPA rather than conjugation to polar compounds.

2.6 Effects of endogenous AbA on plant growth and development during drought stress.

Cell expansion and division are the most sensitive of plant growth processes to water stress (Hsiao, 1973). This is especially important during the vegetative phase of plant growth when expansion of leaves and stems of growing plants is needed for photosynthetic area. Turgor pressure is crucial in cell expansion since a physical force is needed for cell expansion. Turgor pressure is also the crucial component for inducing AbA synthesis (Pierce and Raschke, 1980) indicating that AbA during water stress inhibits the expression of full turgor and, hence, inhibits cell expansion and growth. Quarrie and Jones (1977) showed that AbA applied to the transpiration stream in young unstressed wheat plants produced the same effects as drought stress in that smaller leaves and smaller mean cell size were obtained. Quarrie (1982) also showed the same effects when AbA was injected into the leaf sheaths of older, unstressed spring wheat plants which significantly reduced the flag leaf area by more than half as compared to the controls. However, Hall and McWha (1981) showed that, although daily application of AbA to growing wheat plants

decreased the total dry weight and area of all leaves, the area of green, living leaves and the dry weight were not significantly affected by AbA treatment.

In wheat seedlings, water stress not only affected cell elongation in the coleoptile and primary leaves but also significantly decreased its total protein concentration (Barassi et al., 1980). Dhindsa and Cleland (1975) showed in Avena coleoptiles that both water stress and AbA inhibited the rate of protein synthesis. Independent studies by Scott et al. (1979) and, Quarrie and Henson (1981) indirectly showed the inhibiting effect of AbA on protein synthesis. Scott et al. (1979) investigated the influence of physiological age of a tissue on the response of its polyribosome population to water stress. Polyribosome population is a measure of the protein synthetic potential of a tissue. They suggested that reductions in polyribosome population observed in growing tissues of wheat leaves are a direct result of reductions in growth during water stress. Physiologically older tissues, however, do not show loss of polyribosomes during water stress. Quarrie and Henson (1981) showed that the amount of AbA accumulated in response to water stress decreased as the physiological age of the leaf tissue increased. Protein synthesis capacity is affected in tissues where AbA levels are enhanced.

Drought stress can markedly influence the length of the developmental stages. Angus and Moncur (1977), and Meyers and Green (1980) showed that mild stress in spring wheat plants hastened the interval between floral initiation and anthesis

while severe stress delayed it. Quarrie (1982) showed that treating unstressed spring wheat plants with AbA hastened ear emergence and anthesis significantly implying the involvement of AbA in influencing the length of developmental stages during water stress.

Drought at various stages of development affects the various yield components of cereals (Begg and Turner, 1976). Quarrie (1982) showed that in unstressed wheat plants treated with AbA, there was a significant reduction in the tiller number per plant, ear number per plant, spikelet number per ear and the 1000 grain weight. These reductions in yield components may be explained by a redistribution of assimilates affected by increased levels of AbA (Davies and Mansfield, 1982). Karmoker and Van Steveninck (1979) showed that AbA applied to roots of bean seedlings promoted redistribution of photosynthesis to the roots. Watts et al. (1981) confirmed this finding by showing that AbA increased the overall root:shoot ratio in seedlings of Capsicum annuum, Commelina communis and maize. There are however conflicting reports on the redistribution of assimilates during grain filling as affected by AbA. Increases in endogenous AbA levels in wheat grain (McWha, 1975) and barley grain (Goldbach and Michael, 1976) resulted in increases in dry matter accumulation in the grains. Dewdney and McWha (1979) further showed that AbA applied to wheat grains enhanced assimilate movement from the flag leaf towards the ear. However, Wagner (1974) showed that barley grain imported less assimilate when AbA was supplied to

detached culms. More recently, King and Patrick (1982) found that applied AbA failed to show promotory effects on assimilate accumulation by wheat grains.

Although several studies have been done on phasic changes in growth and development during water stress (Angus and Moncur, 1977; Meyer and Green, 1980; Sionit et al., 1980; Murty and Ramakrishnayya, 1982; Teare et al., 1982; Hockman, 1982), only scant information is available relating phasic changes in AbA levels to water stress. Phasic changes in AbA levels during drought stress were studied by Ilahi and Dorffling (1982). In their study, stress was imposed at three stages of maize development, the one, two and three month stage of growth. They showed that the maximal levels of AbA were higher and were reached earlier in one-month-old plants than in two or three-month old plants. After 16 days of stress, the levels of AbA in the one-month-old plants returned to pre-stressed levels while in two and three-month-old plants they remained at enhanced levels. In terms of AbA sensitivity, the one-month-old plants were apparently the most sensitive stage. Hockman (1982), studied the effect of water stress and phasic development from a different sensitivity, that of yield. He subjected wheat plants to water stress at three stages of development, tillering to anthesis, booting to grainfilling and during grainfilling, and found that the most sensitive stage to drought in terms of yield was the booting to grainfilling stage in which stress reduced yield by 36%. Sionit et al. (1980) found that of the three stages of development in which stress was imposed, seventh leaf, early

anthesis and early dough stages, water stress of -2.5 MPa at all three stages reduced seed yield in wheat but stress applied during early anthesis stage produced the smallest and least number of seeds. Teare et al. (1982) studied the changes in water status during water stress at four different stages of development in wheat i.e. the fourth leaf stage, the seventh leaf stage, anthesis stage and the soft dough stage. The soft dough stage appeared to be most sensitive in terms of changes in leaf water potential and osmotic potential but least sensitive in terms of pressure potential, indicating that osmotic adjustment is occurring at this stage. They also showed that the leaf water potential required to close stomata decreased with growth. Of the five main stages of rice growth viz, seedling stage, tillering stage, booting and flowering stage and grain ripening stage, the most vulnerable stage to moisture stress is the seedling stage (Murty and Ramakrishnayya, 1982).

The fact that AbA accumulates during drought stress has lead research workers to determine the association between AbA content and drought resistance and to use this association as a screening tool for drought resistant varieties. Larque-Saavedra and Wain (1976) showed that tolerant varieties of maize and sorghum contain substantially more AbA during drought stress than less tolerant varieties. Kannangara et al. (1982b) also showed similar findings in two sorghum varieties differing in drought tolerance capacities. A positive correlation has also been shown in millet by Henson et al.

(1981). However, this is not the case in wheat (Quarrie, 1981) and rice (Henson, 1980) in which a negative relationship between drought-induced AbA accumulation and drought resistance was observed. In all these studies, drought resistance was defined from a physiological point of view which may not be relevant to the plant breeder. Tompkins (1980) studied the relationship between AbA accumulation during drought stress and wheat yield of several genotypes and found that the highest yields were obtained from genotypes with moderate levels of AbA in their leaves.

The genetic variation in drought-induced capacity for AbA accumulation in wheat, rice and pearl millet and its high heritability especially in wheat and millet (Austin et al., 1982) has sparked off a race towards developing drought resistant varieties by means of genetic manipulations.

2.7 Summary

A strong association between AbA and water stress in plants has been demonstrated. Generally a negative relationship has been shown between leaf water potential and AbA levels. However, diurnal changes in AbA in field grown plants did not always conform to this relationship. Possible reasons for this have been discussed.

The primary site of action of AbA is in the guard cells. Although only a fraction of the existing AbA is required to induce stomatal closure, it is possible that the additional AbA produced during stress helps the plants during recovery

and for further episodes of stress. The site of synthesis of AbA is a matter of controversy, although recent evidence points to its synthesis largely in the cytosol. Changes in endogenous levels of AbA during stress have been used as a screening tool for drought resistance in cereal plants. Significant differences in endogenous levels in AbA during drought stress have been noticed among the varieties of wheat, maize and rice seedlings. No studies have been made to determine if these differences are significant at various stages of the growth and development of these cereals. Such a study might reveal the appropriate stage at which differences in endogenous AbA levels can be used in screening for drought resistance.

3. MATERIALS AND METHODS

3.1 Plant cultivation

Four pre-germinated seeds of a Mexican semi-dwarf wheat cultivar, Pitic 62, were sown per pot which was 15 cm in diameter and contained a 1:1 mixture of sand and clay-loam soil and 2 g. of 11:51:0 fertilizer. One week after planting, the stands were thinned to two uniformly growing seedlings per pot.

The design of the experiment was a randomized complete block. The pots were irrigated daily and the positions within each of the three replicates were changed once a week. Another application of fertilizer was added prior to the booting stage. The plants were grown under controlled environmental conditions. A 16-hour photoperiod was provided. The light source consisted of a combination of fluorescent and incandescent lamps which provided a total irradiance of $110-120 \text{ Wm}^{-2}$ at plant height as measured with a quantum sensor (Li-Cor Inc., Lincoln, NE., USA). The relative humidity averaged $40\% \pm 10\%$ while temperature was maintained at 20°C .

3.2 Drought treatment

Groups of 12 pots per replicate were subjected to water stress by withholding irrigation at the commencement of each of the following stages of development:

Stage No.	Stage of Development*	Days after planting
1	Tillering	28
2	Stem elongation	39
3	Booting	56
4	Inflorescence emergence	63
5	Milk development	82

(* as described by Zadoks et al., 1974)

Groups of six pots per replicate, treated as controls, received a normal irrigation schedule. For each of the stages, four stressed plants per replicate (total of 12 plants for 3 replicates) were harvested daily up to five days after the stress began. Control plants were harvested on days coinciding with the first, third and fifth days of stress.

3.3 Sampling procedure

3.3.1 Measurement of stomatal resistance

Measurements commenced four hours after the start of the photoperiod. For each plant harvested, the stomatal resistance

was measured using an automatic dynamic-diffusion porometer (Delta-T Devices, Cambridge, U.K.) on the adaxial (R_u) and abaxial (R_l) surfaces of the third fully collared leaf from the apex of the main tiller. The mean R_u or R_l values from four samples were calculated for each replicate. The maximum stomatal resistance for either surface was taken at 30 s/cm (Teare et al, 1982). Total stomatal resistance (R_T) was obtained from the equation, $R_T = \frac{R_u \times R_l}{R_u + R_l}$.

3.3.2 Measurement of total leaf water potential, ψ_T , osmotic potential, ψ_π , and pressure potential, ψ_p .

The third fully collared leaf from the apex of the main tiller was covered with a plastic sheath, excised (Turner and Long, 1980) and its water potential (ψ_T) measured using a pressure chamber (Model 600, PMS, Corvallis, Oregon, USA) modified to accomodate four leaves at a time. Oxygen-free nitrogen was used as a gas-pressure source. The four excised leaves, still in the plastic sheaths, were inserted into the clamping device of the pressure chamber so that the cut ends were protruding to the outside. The pressure of the gas was increased at a slow but constant rate and the pressure when sap first wetted the cut surfaces of the leaves, as observed through a magnifying glass, was recorded. A mean of four readings were obtained per replicate.

After removal from the pressure chamber, each leaf was inserted into a 3-ml plastic disposable syringe. These

syringes were sealed in plastic bags and frozen in liquid nitrogen and stored in a -14°C freezer. They were later thawed for between half an hour and one hour and the expressed sap was used to measure the osmotic potential (ψ_{π}) using a vapor-pressure osmometer (Wescor Inc., Logan, Utah, USA). The pressure potential (ψ_p) was obtained from the equation,

$$\psi_p = \psi_t - \psi_{\pi}.$$

Turgor maintenance under drought stress due to solute accumulation was analysed by the method of Morgan (1980). This method involved comparing the observed response with that expected from an ideal osmometer (Van't Hoff's line) in which concentration of solute is changed only by the gain or loss of water from the cell.

For an ideal osmometer,

$$\psi_{\pi} = \psi_{\pi_o} V_o / V$$

which can be approximated to

$$\psi_{\pi} = \psi_{\pi_o} \text{RWC}_o / \text{RWC}$$

where V_o is the osmotic volume and, ψ_{π_o} and RWC_o are the osmotic potential and relative water content respectively, at full turgor.

3.3.3 Measurement of relative water content (RWC)

The fourth fully collared leaf from the apex of the main tiller was excised and used wholly in determining the relative water content (Barrs and Weatherley, 1962). After determining its fresh weight, turgid weight was found by placing the cut

ends of four leaves per replicate in test-tubes containing water. The test-tubes were kept covered at room temperature and left for 16-18 hours. The submerged ends of the leaves were blotted rapidly and thoroughly before weighing. The leaves were then transferred to an oven at 100°C for 24 hours and their dry weights were determined. The RWC was calculated according to the formula below:

$$\text{RWC} = \frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Turgid wt.} - \text{Dry wt.}} \times 100\%$$

The mean of RWC values from four leaves per replicate was found.

3.4 AbA analysis

3.4.1 Extraction and purification

The first two fully expanded leaves from the apex of the main tiller at each stage were excised and frozen in liquid nitrogen and stored at -70°C. The tissues were freeze-dried and about 100-200 mg of the freeze-dried tissues per replicate was extracted for analysis of AbA (Durley *et al.*, 1982).

The tissue was homogenized in pre-cooled (4°C) methanol-water (80:20; 50 mL), which contained 200 mg/L of the antioxidant, sodium diethyldithiocarbamate. The homogenised tissue was stirred and the residue resuspended in methanol-water (80:20) for 2 hours. This mixture was again filtered and the two filtrates combined. The filtrate was evaporated in vacuo and the residue taken up by water:methanol (70:30). This

solution was centrifuged at 12,100g for 40 minutes. The supernatant was carefully removed and evaporated in vacuo.

The residue was again taken up at pH 3.0 and extracted (4 times) with diethyl ether. The residue from the evaporated phase was dissolved in 1 mL methanol-diethyl ether (1:1) and treated dropwise with concentrated ammonium hydroxide solution, with shaking, until the ammonia was in excess (6-8 drops). The solution was evaporated in vacuo. The ammonium salts prepared were dissolved in 2 mL of water and chromatographed on polyvinylpyrrolidone (PVP) columns (2cm x 1.5cm). The columns were eluted with distilled water containing sodium diethyldithiocarbamate (200 mg/L). The first 25 mL were collected, adjusted to pH 3.0, extracted (3x) with diethyl ether which was evaporated.

3.4.2 High-performance liquid chromatography of extracts

The extracts from the PVP treatment were dissolved in methanol (1 mL) and transferred to 1-dram vials and evaporated using oxygen-free nitrogen. The residue was taken up with 160 μ L of water-methanol (1:1) and injected onto a 25 x 0.46 cm I.D. Beckman Ultrasphere ODS column. The mobile phase was a linear gradient of water-methanol-acetic acid starting with composition (30:70:0.5). The flow rate was 1.0 mL/min. over a period of 50 minutes. The fraction corresponding to the elution time of standard AbA samples was collected and evaporated. The residue was taken up with methanol and evaporated in half-dram vials using oxygen-free nitrogen.

AbA samples were analysed on a 25 x 0.46 cm I.D. Beckman Ultrasphere Si (analytical) column. The mobile phase was chloroform-acetonitrile-acetic acid (96:3:1) and the flow-rate was 1.0 mL/min. Samples were dissolved in 50 μ L of the mobile phase and either 5 or 10 μ L of this were injected into the analytical column. Peaks were detected by UV absorption at 254 nm and transcribed onto a recorder. The hormone levels were estimated by measuring peak heights. The recovery efficiency for AbA was 75%.

3.5 Measurement of dry matter content of total above-ground parts

The rest of the above-ground parts of the plants were used for measurement of shoot dry matter content. The total above-ground parts were harvested and then transferred to an oven, set at 100°C for 24 hours. Their dry weights were then determined. The mean dry matter content from four samples per replicate was found.

The relative growth rate (RGR) was calculated for each stage of development from the following equation,

$$\text{RGR} = \frac{W_2 - W_1}{W_1}$$

where, W_1 = Dry matter (g.) at onset of stress period,

W_2 = Dry matter (g.) at end of stress period.

3.6 Estimation of soil water content

Two soil samples taken daily from the centre of each pot were weighed, oven dried at 100°C for 48 hours, then reweighed to determine the soil moisture content. Soil water content was calculated according to the following equation;

$$\text{Soil water content (\%)} = \frac{\text{Wet wt.} - \text{Dry wt.}}{\text{Wet wt.}} \times 100\%$$

The mean of soil water contents from four samples per replicate was determined.

4. RESULTS

4.1 Changes in total leaf water potential (ψ_T) and soil water content and its relationship

There were significant decreases in soil water content over the period of stress for all stages of development (Table 4.1). Using differences in soil water content between the commencement of stress and the end of the stress period as an indicator of the severity of stress, it can be seen from Table 4.2 that the first four stages experienced more severe stress than the milk development stage.

In the tillering, inflorescence emergence and milk development stages, there was a gradual drop in ψ_T as the soil water content dropped (Fig. 4.1). In the stem elongation and booting stages decreases in soil moisture did not affect ψ_T until about 7% and 5% respectively, after which ψ_T dropped sharply.

Less moisture remained in the soil after drought stress periods at the stem elongation, booting and inflorescence emergence stages than at the tillering or milk development stages indicating again that greater stress occurred in the former group (Fig 4.2a). There were no significant changes in soil water content of the controls at all stages of development (Table 4.1; Fig. 4.2b).

Table 4.1 Mean squares from the analyses of variance of total leaf water potential (Ψ_T), leaf osmotic potential (Ψ_π), leaf pressure potential (Ψ_p), relative water content, (RWC), ABA and soil water content among days of stress (df=4, error df=10) and among (non-stressed) control days (df=2, error df=5) at five stages of wheat development.

Stage of development	Source of variation	Mean squares					
		Total leaf water potential (Ψ_T)	Leaf osmotic potential (Ψ_π)	Leaf pressure potential (Ψ_p)	RWC	ABA	Soil water content
Tillering	Stress days	11.65**	0.38	9.99**	7.82**	31644.18**	25.52**
	Error	0.65	0.15	0.74	0.89	690.13	0.69
	Control days	0.19	1.08	1.47	1.05	676.00	12.05
	Error	0.50	0.35	0.88	1.33	571.33	2.56
Stem elongation	Stress days	225.23**	63.04**	50.71**	1408.19**	47898.00**	18.03**
	Error	3.14	3.45	2.42	9.56	276.00	0.37
	Control days	0.25	0.08	0.07	2.06	54.33	1.33
	Error	2.11	0.35	0.98	3.00	130.89	1.50
Bootling	Stress days	471.10**	215.88**	52.34**	387.89**	58970.00**	13.37**
	Error	9.55	17.49	8.38	39.63	993.60	1.68
	Control days	0.11	0.33	0.35	43.44	47.44	8.70
	Error	0.70	0.89	1.50	4.34	39.11	2.42
Inflorescence emergence	Stress days	420.98**	274.90**	21.41*	769.73**	53914.00**	11.01**
	Error	7.62	11.43	8.29	25.38	270.93	0.79
	Control days	2.45	1.55	0.22	1.29	142.33	8.50
	Error	0.73	0.35	1.45	1.49	160.22	3.05
Milk development	Stress days	64.93**	35.27**	11.51*	107.53**	137291.00**	5.40**
	Error	3.96	1.72	2.90	18.55	10689.00	0.50
	Control days	10.68	4.17	2.49	22.44	8463.00	8.09
	Error	6.52	2.47	1.10	6.79	3661.33	3.54

* P = 0.05

** P = 0.01

Table 4.2 Depletion of soil water during drought stress at five stages of wheat development.

Stage of Development	% Depletion of Soil Water*
1. Tillering	63
2. Stem elongation	62
3. Booting	69
4. Inflorescence emergence	66
5. Milk development	45

*
$$\frac{\text{Initial soil water content} - \text{final soil water content}}{\text{Initial soil water content}} \times 100$$

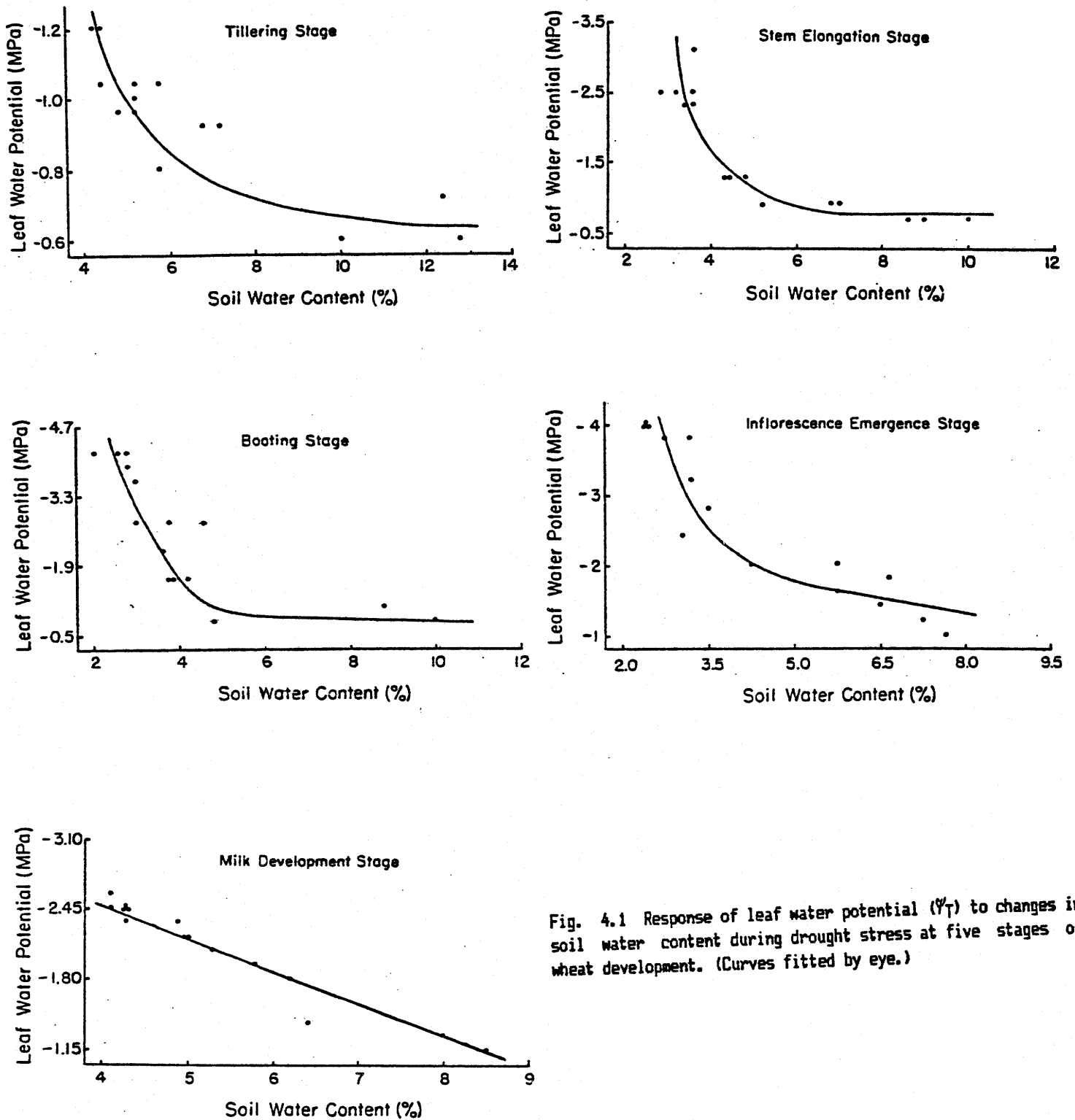


Fig. 4.1 Response of leaf water potential (Ψ_L) to changes in soil water content during drought stress at five stages of wheat development. (Curves fitted by eye.)

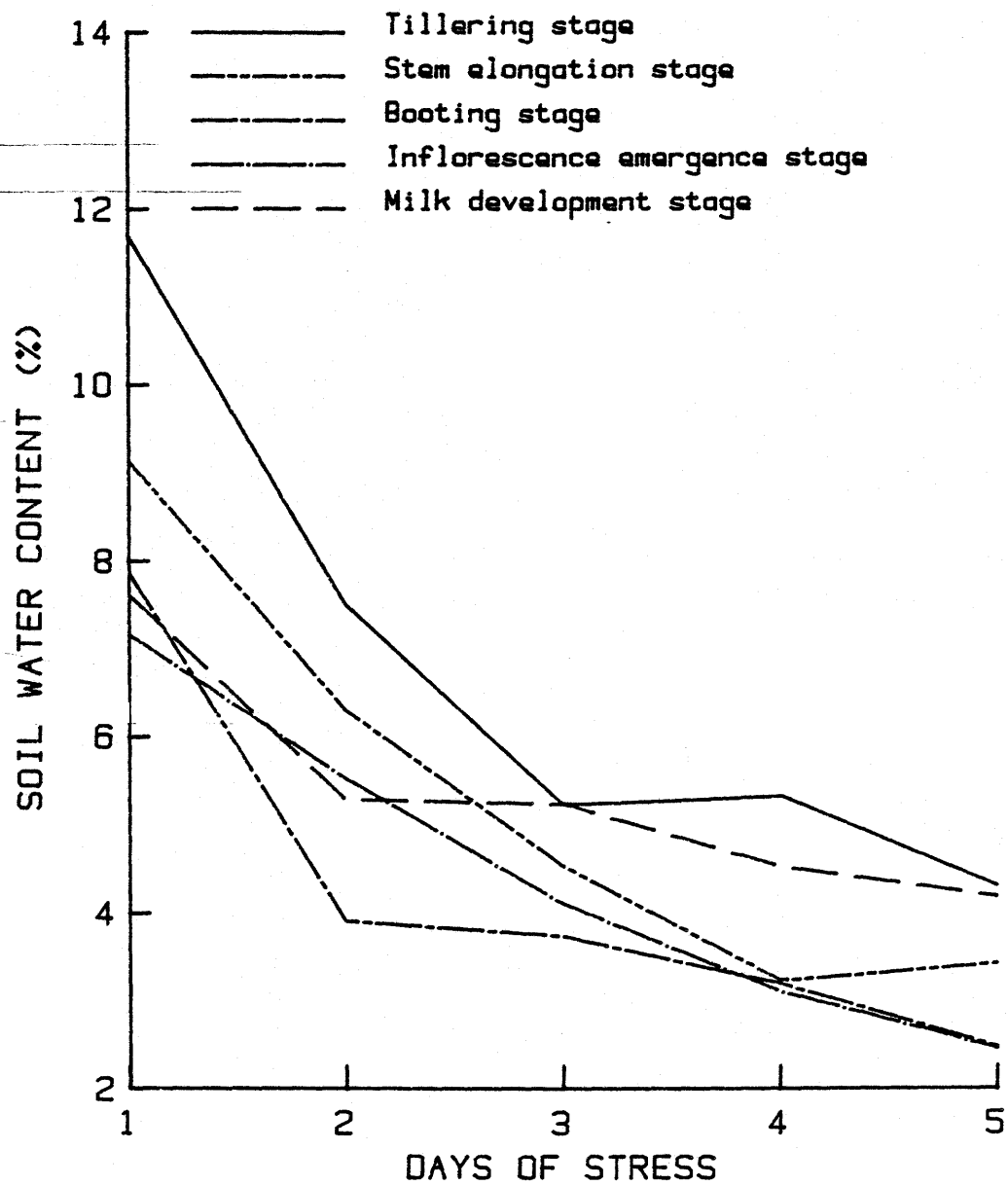


Fig. 4.2a Depletion of soil water content during drought stress at five stages of development.

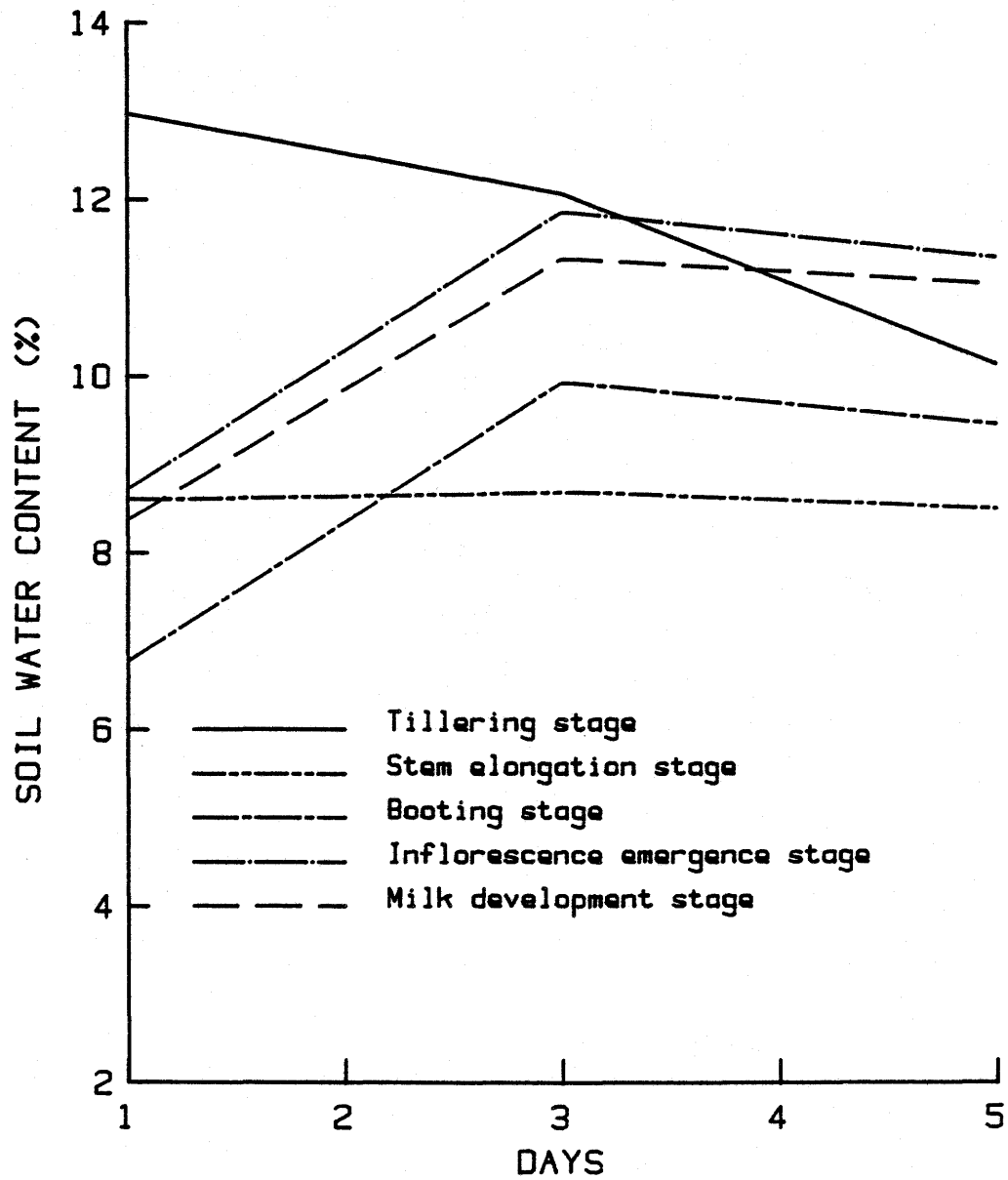


Fig. 4.2b Depletion of soil water content in control wheat plants at five stages of development.

4.2 Effects of stress on the total leaf water potential (ψ_T)

There were significant reductions in the total leaf water potential (ψ_T) over the period of stress at all stages of development (Table 4.1). High correlations were obtained between ψ_T and soil water content at all stages, indicating that as stress increased, ψ_T decreased (more negative) (Table 4.3). There were no significant differences in the ψ_T for the control treatments at all stages of development (Table 4.1).

Sharp decreases in the ψ_T over the stress period were observed in the stem elongation, booting and the inflorescence emergence stages (Fig. 4.3). But in the tillering and the milk development stages decreases were less steep. In both these stages, there was a significant reduction in the ψ_T on the second day of stress. The next significant drop in ψ_T was observed on the fourth day of stress for the tillering stage and on the fifth day of stress for the milk development stage (Table 4.4). In both these stages, the soil water contents on the last day of stress were 4.30% and 4.18% respectively, which were not as stressful as on the other three stages.

4.3 Response of osmotic potentials, ψ_π , and pressure potentials, ψ_P , to changes in relative water contents (RWC) during water stress

All stages except the tillering stage showed significant reductions (more negative) in the ψ_π over the period of stress (Table 4.1). Pressure potentials, ψ_P , were found to decrease

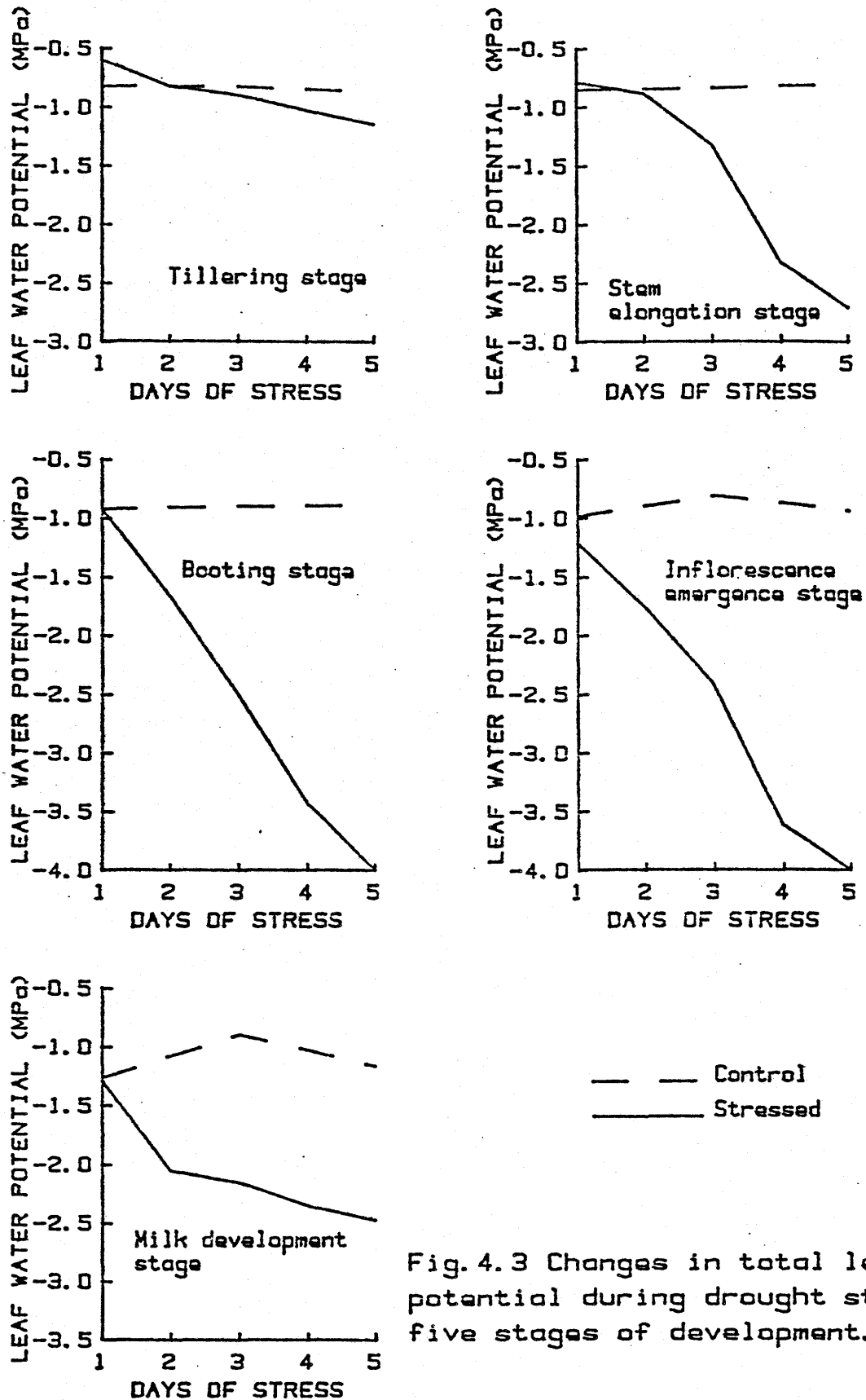


Fig. 4.3 Changes in total leaf water potential during drought stress at five stages of development.

Table 4.3 Correlation coefficients between total leaf water potential (Ψ_T), and soil water content during drought stress at five stages of wheat development.

Stage of development	Correlation coefficient (r values)
1. Tillering	0.873**
2. Stem elongation	0.813**
3. Booting	0.748**
4. Inflorescence emergence	0.928**
5. Milk development	0.974**

P = 0.01

Table 4.4 Comparison of daily means of total leaf water potential, (Ψ_T), leaf osmotic potential, (Ψ_π), leaf pressure potential, (Ψ_p), relative water content, (RWC), ABA and soil water content during drought stress at five stages of wheat development.

Variable	Days of stress					Days of stress					
Stage 1	1	2	3	4	5	Stage 4	1	2	3	4	5
Ψ_T (MPa)	-0.63a	-0.83b	-0.90bc	-1.03cd	-1.15d	Ψ_T (MPa)	-1.23a	-1.77b	-2.42c	-3.62d	-4.00d
Ψ_π (MPa)	-1.12a	-1.06a	-1.11a	-1.11a	-1.16a	Ψ_π (MPa)	-1.46a	-1.84ab	-2.43b	-3.39c	-3.67c
Ψ_p (MPa)	0.48a	0.23b	0.21b	0.07bc	0.01c	Ψ_p (MPa)	0.23a	0.07b	-0.09c	-0.41d	-0.33e
RWC (%)	98.97a	98.10ab	98.50ab	97.10b	94.90c	RWC (%)	96.82a	91.14a	74.50b	67.37bc	58.66c
ABA (ng./g. dry wt.)	158a	210ab	219b	294c	429d	ABA (ng/g dry wt.)	266a	351b	365b	534c	586c
Soil water content (%)	11.70a	7.50b	5.23c	5.33c	4.30c	Soil water content (%)	7.16a	5.52b	4.10bc	3.01c	2.45c
Stage 2	1	2	3	4	5	Stage 5	1	2	3	4	5
Ψ_T (MPa)	-0.79a	-0.89a	-1.32b	-2.33c	-2.71d	Ψ_T (MPa)	-1.29a	-2.06b	-2.16bc	-2.36bc	-2.48c
Ψ_π (MPa)	-1.93a	-1.33a	-1.35a	-1.87b	-2.07b	Ψ_π (MPa)	-1.52a	-2.33b	-2.10b	-2.22b	-2.35b
Ψ_p (MPa)	0.25a	0.24a	0.27a	-0.47b	-0.64b	Ψ_p (MPa)	0.22a	0.26a	-0.06ab	-0.14b	-0.13b
RWC (%)	98.90a	88.03b	79.00c	57.07d	46.63e	RWC (%)	91.11a	83.70ab	81.93b	78.17b	75.46b
ABA (ng/g dry wt.)	220a	256b	340c	482d	502d	ABA (ng/g dry wt.)	420a	643b	849c	903c	923c
Soil water content (%)	9.13a	6.30b	4.53c	3.23c	3.43c	Soil water content (%)	7.61a	5.28b	5.23b	4.52b	4.18b
Stage 3	1	2	3	4	5						
Ψ_T (MPa)	-0.93a	-1.67b	-2.51c	-3.43d	-4.00e						
Ψ_π (MPa)	-1.15a	-1.60ab	-2.00bc	-2.76cd	-3.24d						
Ψ_p (MPa)	0.02a	-0.07ab	-0.51bc	-0.67c	-0.76c						
RWC (%)	87.23a	67.97b	64.33b	62.03b	58.11b						
ABA (ng/g dry wt.)	258a	260a	340b	472c	578d						
Soil water content (%)	7.87a	3.90b	3.73b	3.19b	2.47b						

Means followed by same letters within rows indicate no significant difference at $P = 0.05$, according to Duncan's Multiple Range Test.

(more negative) significantly at all stages of development (Table 4.1). There were however no significant decreases in ψ_{π} or ψ_p in the control plants at all stages.

The tillering stage was the least affected by drought stress. Only at the fifth day of stress was the relative water content (RWC) significantly less than on the previous days of stress period (Table 4.4). However, reduction in RWC was greatest over the stress period at the stem elongation stage, where a loss of 52% was observed (Fig 4.4a). No significant reductions in the relative water contents were observed in the control plants at all stages of development (Table 4.1; Fig. 4.4b)

The relationship between ψ_{π} and RWC can be described by the equation $\psi_{\pi} = a + bRWC$. With the exception of the tillering stage, RWC accounted for 54 to 90% of the variance in ψ_{π} (Table 4.5). There were also significant changes in ψ_{π} with respect to RWC in all stages except the tillering stage. The response of ψ_{π} to changes in RWC at the tillering stage showed similar behaviour to that of an ideal osmometer (Van't Hoff's line) indicating no osmotic adjustments occurred (Fig. 4.5)

In the stem elongation stage, a comparison of daily means of ψ_{π} showed that there were slight decreases (non-significant) in ψ_{π} over the first three days while RWC decreased significantly over this period (Table 4.4). These changes in ψ_{π} can be accounted for by the concentration of solutes. However, this could not be adequately explained by

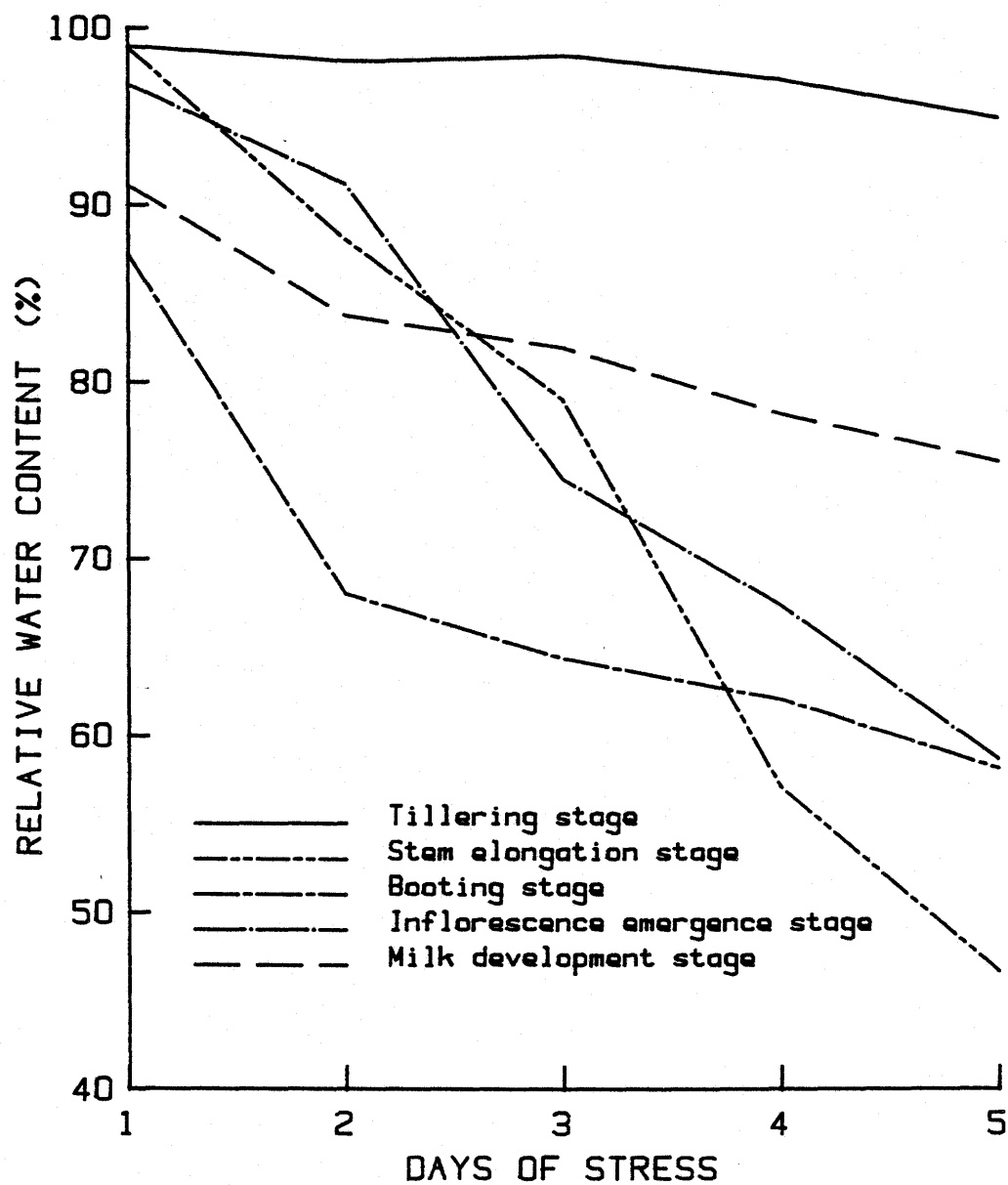


Fig. 4.4a Changes in relative water content during water stress at five stages of development.

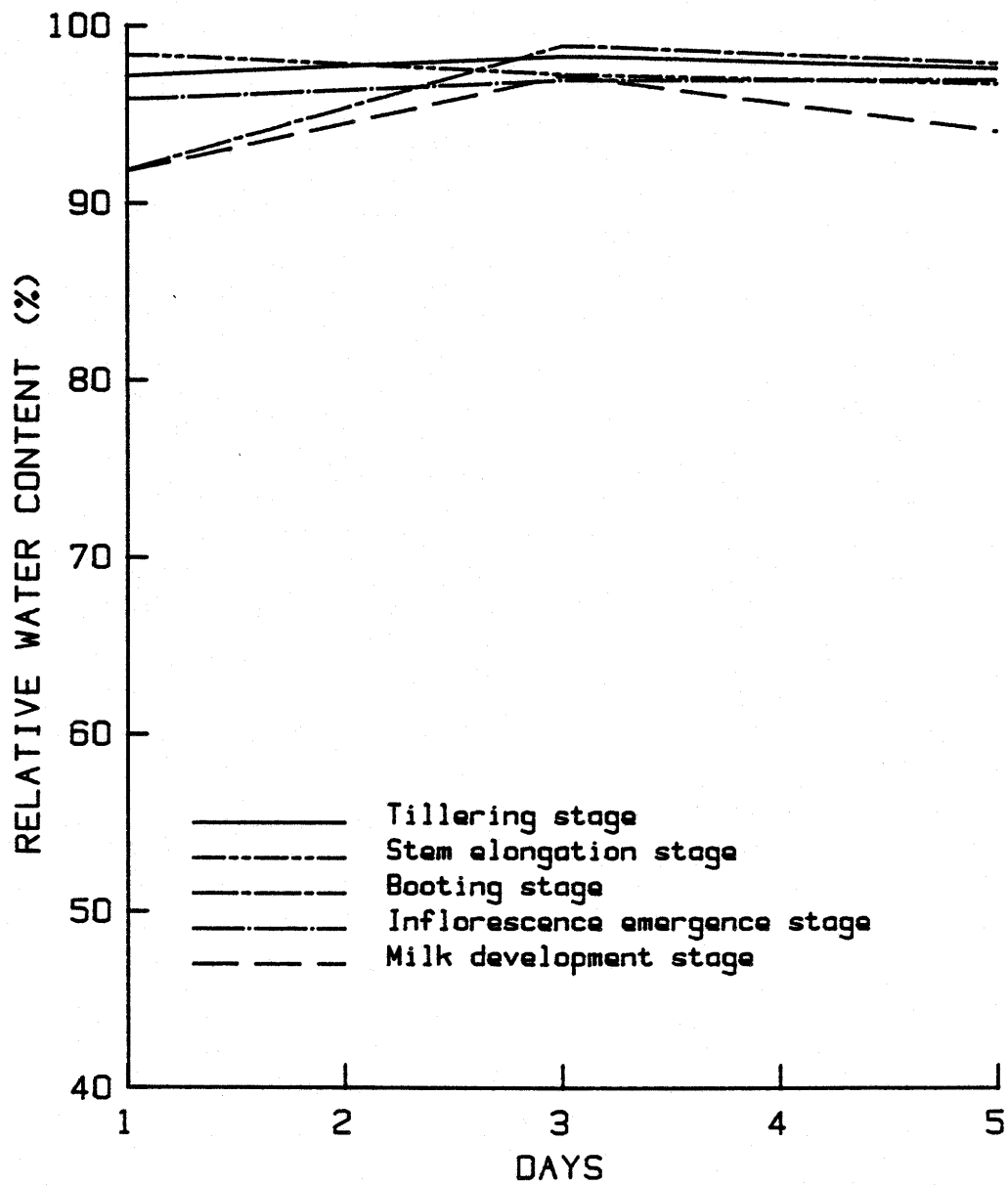


Fig. 4.4b Changes in relative water content in control wheat plants at five stages of development.

Table 4.5 Response of leaf osmotic potential (ψ_{π}) to changes in relative water content (RWC) during drought stress at five stages of wheat development given by the equation, $\psi_{\pi} = a + b\text{RWC}$

Stage of development	Regression coefficients		% variance explained by regression line
	a	b	
Tillering	-2.31	0.0123	13.8
Stem elongation	-3.06	0.0213*	89.9
Booting	-5.98	0.0564*	56.4
Inflorescence emergence	-6.56	0.0516*	70.9
Milk development	-5.26	0.0384*	54.1

* $P=0.05$

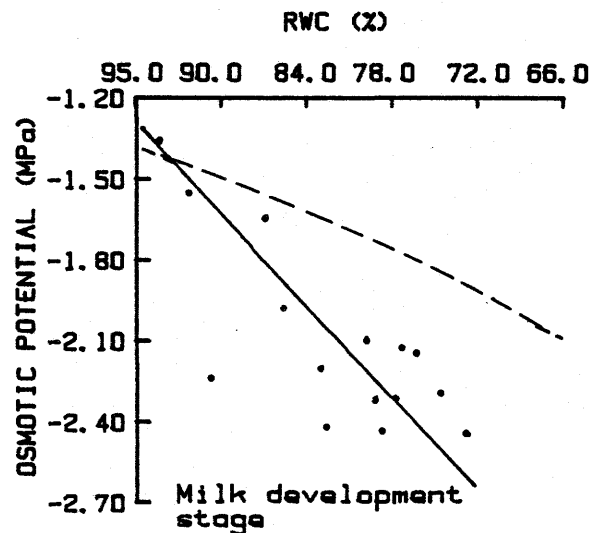
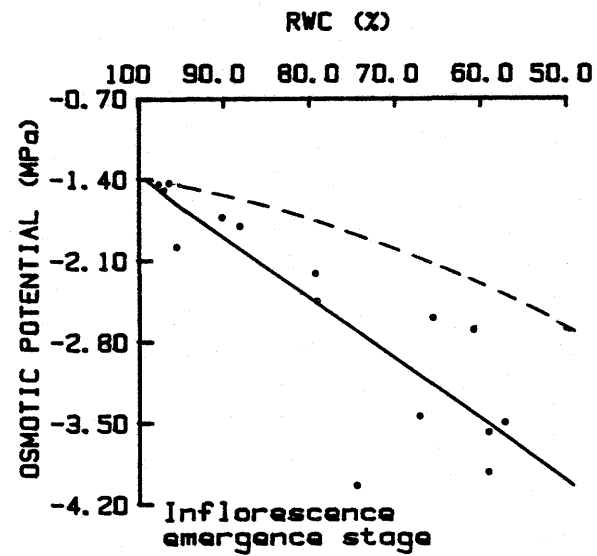
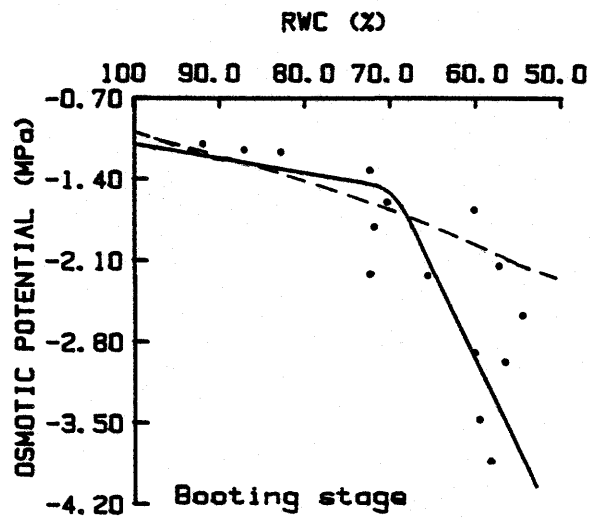
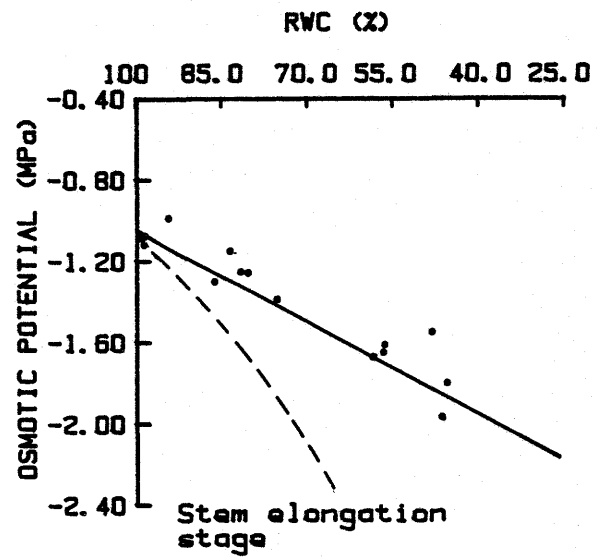
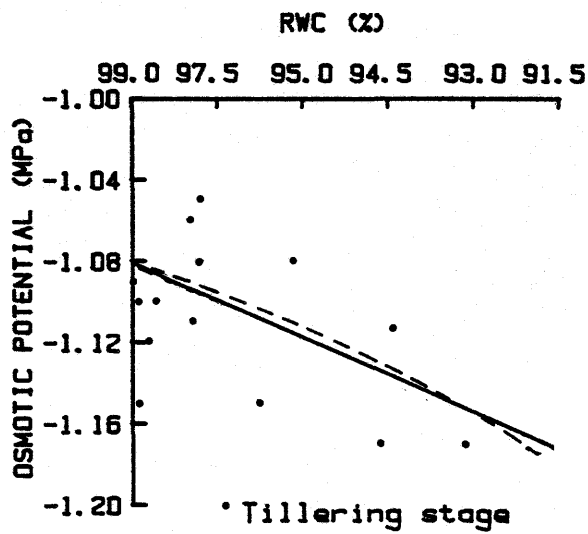


Fig. 4.5 The response of osmotic potential (Ψ_{π}) to changes in relative water content (RWC) during drought stress at five stages of development. The broken lines are the responses of an ideal osmometer (Van't Hoff's lines).

comparing the response to the Van't Hoff's line (Fig 4.5).

At some point in the stress period at the later stages of development (stages 3, 4 and 5) osmotic adjustments occur. In stage 3 (Fig. 4.5) osmotic adjustments occurred when RWC dropped to approximately 70%. In stage 5, there was a decrease in Ψ_{π} from -1.52 MPa to -2.33 MPa after the first day of stress while RWC dropped slightly (non-significantly) (Table 4.4). There were no significant decreases in Ψ_{π} or RWC for the remainder of the stress period. In stage 4 there were significant decreases in both Ψ_{π} and RWC on the third and fifth days of stress (Table 4.4). There was also a significant decrease in Ψ_{π} from the third day to the fourth day of stress while no significant decrease in RWC was observed in that period, suggesting osmotic adjustments were occurring. In all these three stages, the response of Ψ_{π} to changes in RWC deviated very much from the Van't Hoff's line (Fig. 4.5) indicating the occurrence of osmotic adjustments.

4.4 Changes in endogenous AbA levels during water stress

Highly significant increases in AbA levels were observed during drought stress (Tables 4.1 and 4.4) while non-significant changes in AbA levels of control plants were observed at all stages of growth (Table 4.1; Fig. 4.6b). The highest differential in AbA production over the stress period was found in the milk development stage while the least differential in AbA production was found in the tillering

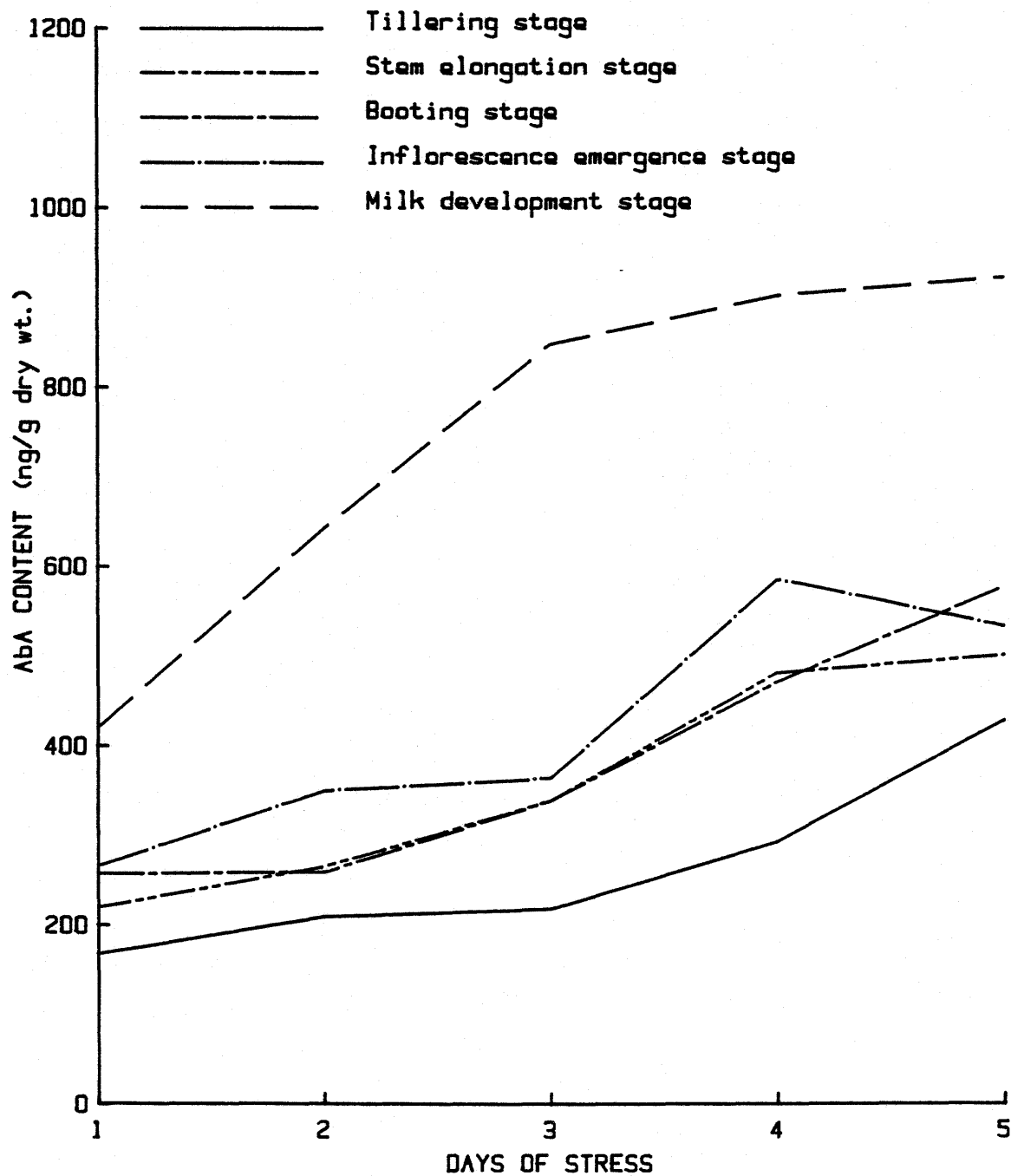


Fig. 4.6a Changes in ABA accumulation during drought stress at five stages of development.

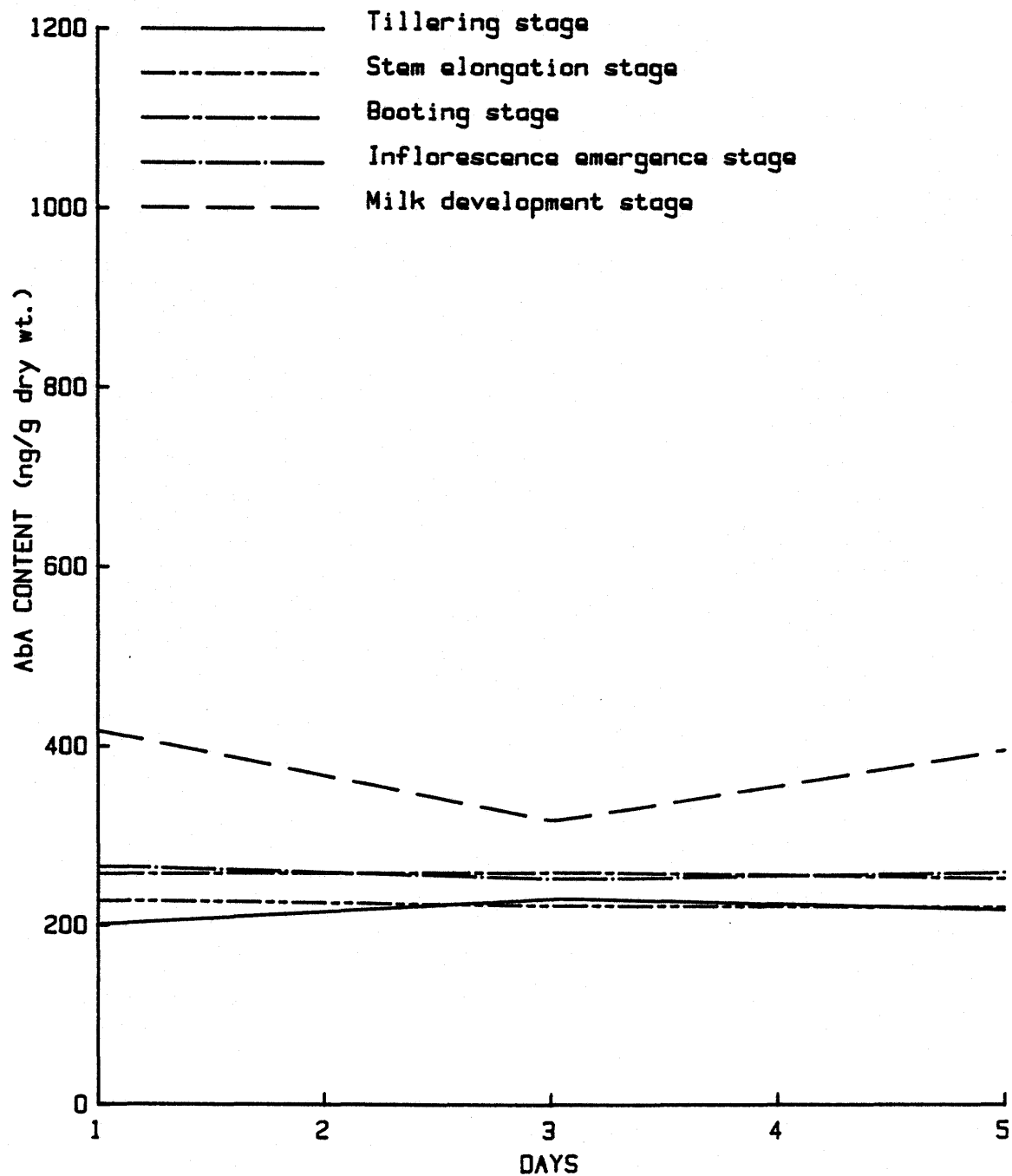


Fig. 4.6b Changes in AbA accumulation in control wheat plants at five stages of development.

Table 4.6 Changes in AbA* accumulation during drought stress at five stages of wheat development.

Stage of development	AbA levels at onset of stress	Maximal AbA levels during stress	Change in AbA levels	Relative increase **
Tillering	168	428	260	2.55
Stem elongation	220	502	282	2.28
Booting	258	578	320	2.24
Inflorescence emergence	266	586	320	2.20
Milk development	420	922	502	2.20

* ng/g dry wt.

**Relative Increase =
$$\frac{\text{Maximal AbA level}}{\text{AbA level at onset of stress}}$$

Table 4.7 Analysis of variance for AbA levels during drought stress.

Source of variation	df	SS	MS	F
Stages of development	4	2030683.0	507671.0	196.47**
Days of stress	4	1111342.0	277836.0	107.52**
Stages x Days	16	207527.0	12970.0	5.02**
Error	50	129197.0	2584.0	
Total	74	3478749.0		

** P = 0.01

stage (Fig. 4.6a; Table 4.6). Drought stress induced more AbA production at later stages of growth.

Analysis of variance showed that there were highly significant differences in AbA production between the stages of development during water stress (Table 4.7). Highly significant differences in AbA levels were also obtained between the days of stress. A highly significant interaction between the days of stress and the stages of development was also shown.

In stage 5, the level of AbA increased significantly over the first two days and then reached a fairly constant level for the remaining period of stress (Fig. 4.6a; Table 4.4). In the remaining stages there was a tendency for AbA to increase moderately over two or three days of stress and then produce large quantities of AbA late in the stress period.

Although the absolute amounts of AbA produced differed greatly between stages, there were very small differences in relative increase in the AbA levels at all stages of development. Relative increases in AbA levels (expressed as a fraction of its initial amount) were in the range of 2.20 to 2.55 (Table 4.6). In contrast to the absolute amounts of AbA produced, the highest relative increase in AbA was observed at the tillering stage where a 2.55-fold increase was detected. Generally the relative increase was smaller at later stages of development.

Correlations between AbA and the parameters of plant water status showed that all four variables viz. ψ_T , ψ_n , ψ_p and

RWC, were significantly correlated with AbA (Table 4.8). ψ_T had consistently higher r values than the other parameters at all stages of development.

The relationship between AbA and ψ_T also shows that in at least three stages there was a ψ_T threshold at which AbA production was triggered (Fig. 4.7). Similar thresholds were observed for ψ_P (Fig. 4.8). A consistency could be established in the ψ_P at which AbA accumulation was induced. The ψ_P threshold range was between 0.13 to -0.25 MPa, clearly indicating that zero or low turgor initiates an increase in AbA content (Table 4.9). Generally, AbA production was triggered at lower water potentials in the later stages of development. The most sensitive stage at which decreases in ψ_T stimulated AbA production seemed to be the stem elongation stage although in the tillering stage AbA production was triggered at a ψ_P which was higher than that of any other stage.

4.5 Plant responses to increases in endogenous AbA levels

4.5.1 Effects of endogenous AbA on stomatal resistance.

Critical total water potentials at which stomatal resistance (R_T) was induced, were detected at four stages of development and generally decreased with advancing stage of development (Fig. 4.9). The inflorescence emergence stage,

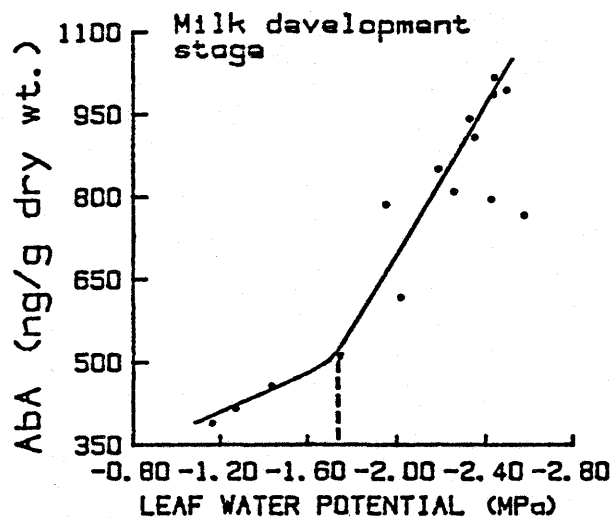
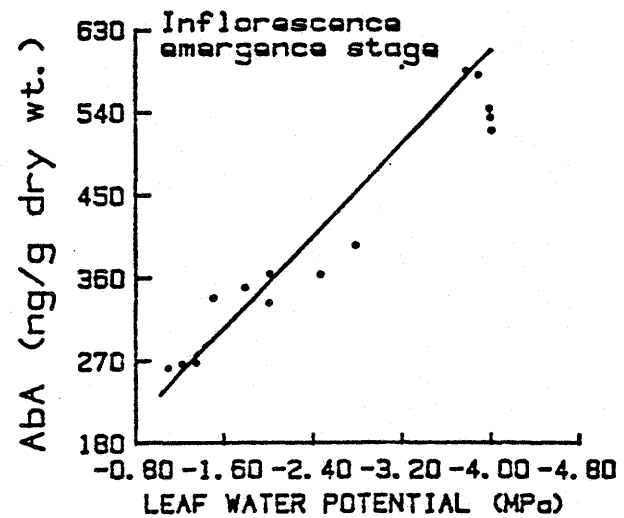
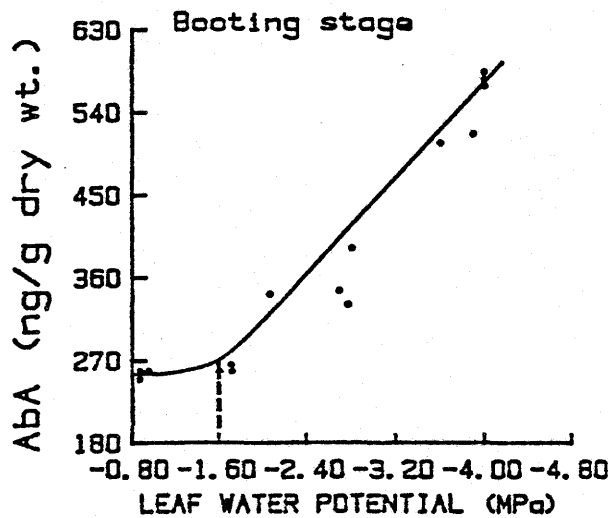
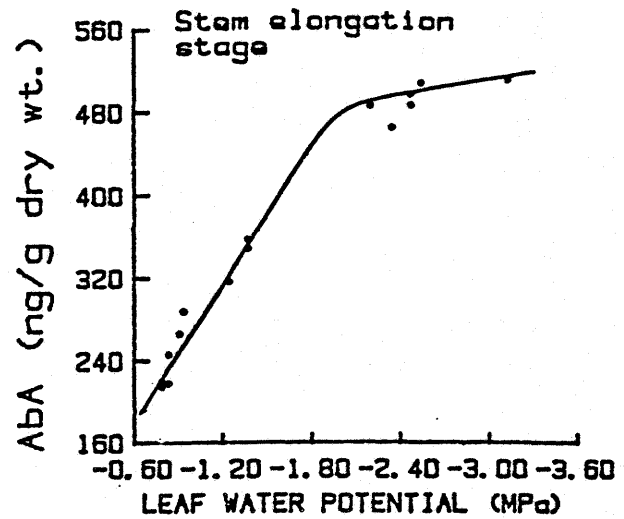
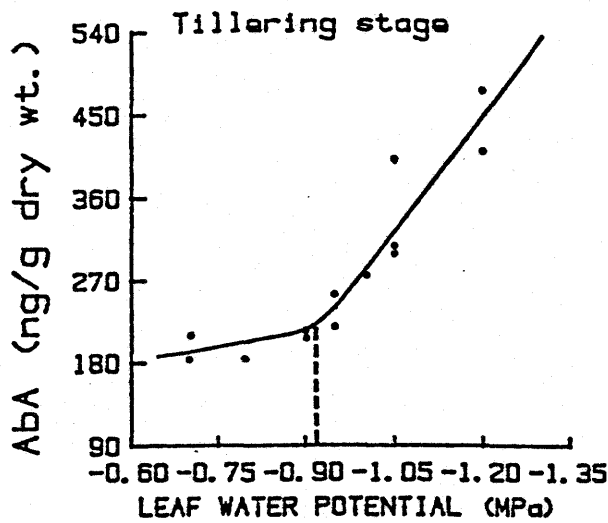


Fig. 4.7 Relationship between AbA and total leaf water potential (Ψ_r) during drought stress at five stages of development. Curves fitted by eye. Dotted lines indicate Ψ_r thresholds for AbA production.

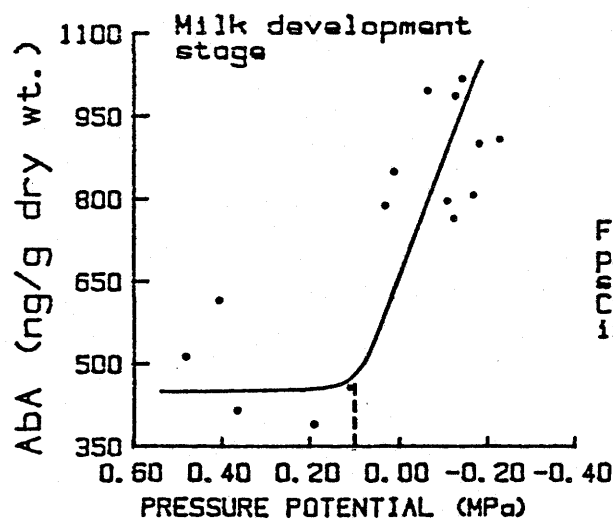
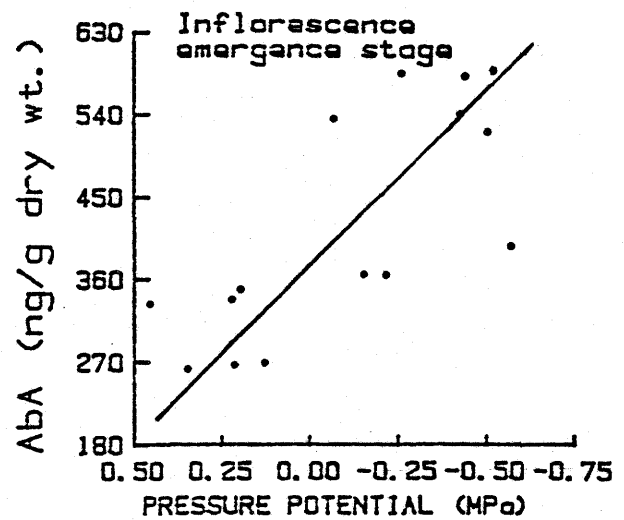
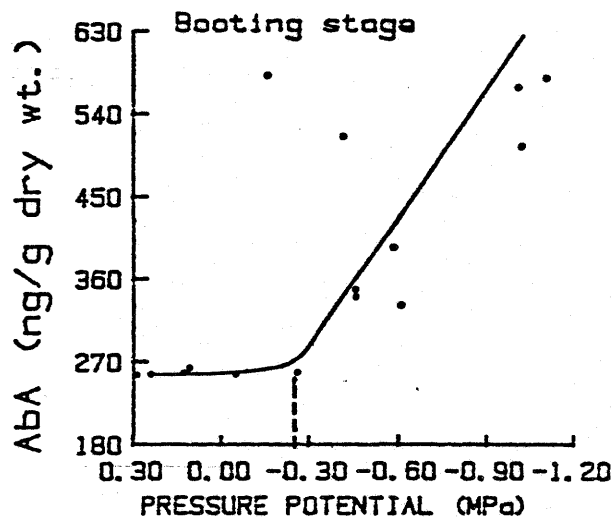
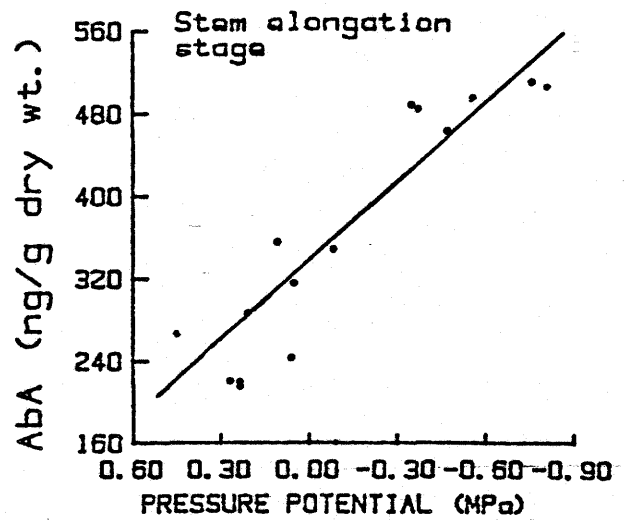
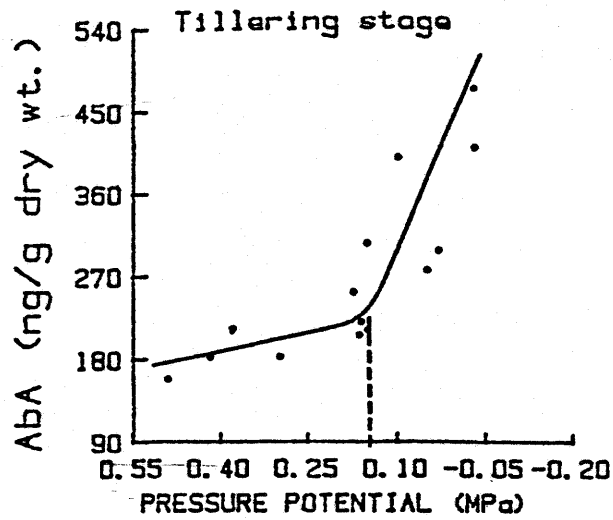


Fig. 4.8 Relationship between AbA and pressure potential (ψ_p) during drought stress at five stages of development. Curves fitted by eye. Dotted lines indicate ψ_p thresholds for AbA production.

Table 4.8 Correlation coefficients between ABA and parameters of plant water status during drought stress at five stages of wheat development.

ABA x	Treatment	Correlation coefficients (r)				
		Tillering stage	Stem elongation stage	Booting stage	Inflorescence emergence stage	Milk development stage
Total leaf water potential	Stress	-0.89**	-0.97**	-0.95**	-0.94**	-0.88**
	Control	-0.62*	-0.86**	-0.16	-0.18	-0.94**
Leaf osmotic potential	Stress	-0.54*	-0.94**	-0.92**	-0.90**	-0.66**
	Control	0.43	-0.87**	0.28	-0.15	-0.88**
Leaf pressure potential	Stress	-0.80**	-0.92**	-0.73**	-0.76**	-0.80**
	Control	-0.63	-0.77**	-0.33	-0.06	-0.91**
Relative water content	Stress	-0.88**	-0.99**	-0.49*	-0.84**	-0.83**
	Control	0.25	-0.43	-0.12	-0.20	-0.46

* P = 0.05

** P = 0.01

Table 4.9 Total leaf water potential (ψ_T), and leaf pressure potential (ψ_P) thresholds for AbA production during drought stress at five stages of wheat development.

Stage of Development	ψ_T (MPa)	ψ_P (MPa)
Tillering	-0.92	0.13
Stem elongation	*	*
Booting	-1.60	-0.25
Inflorescence emergence	*	*
Milk development	-1.75	0.11

* not detectable

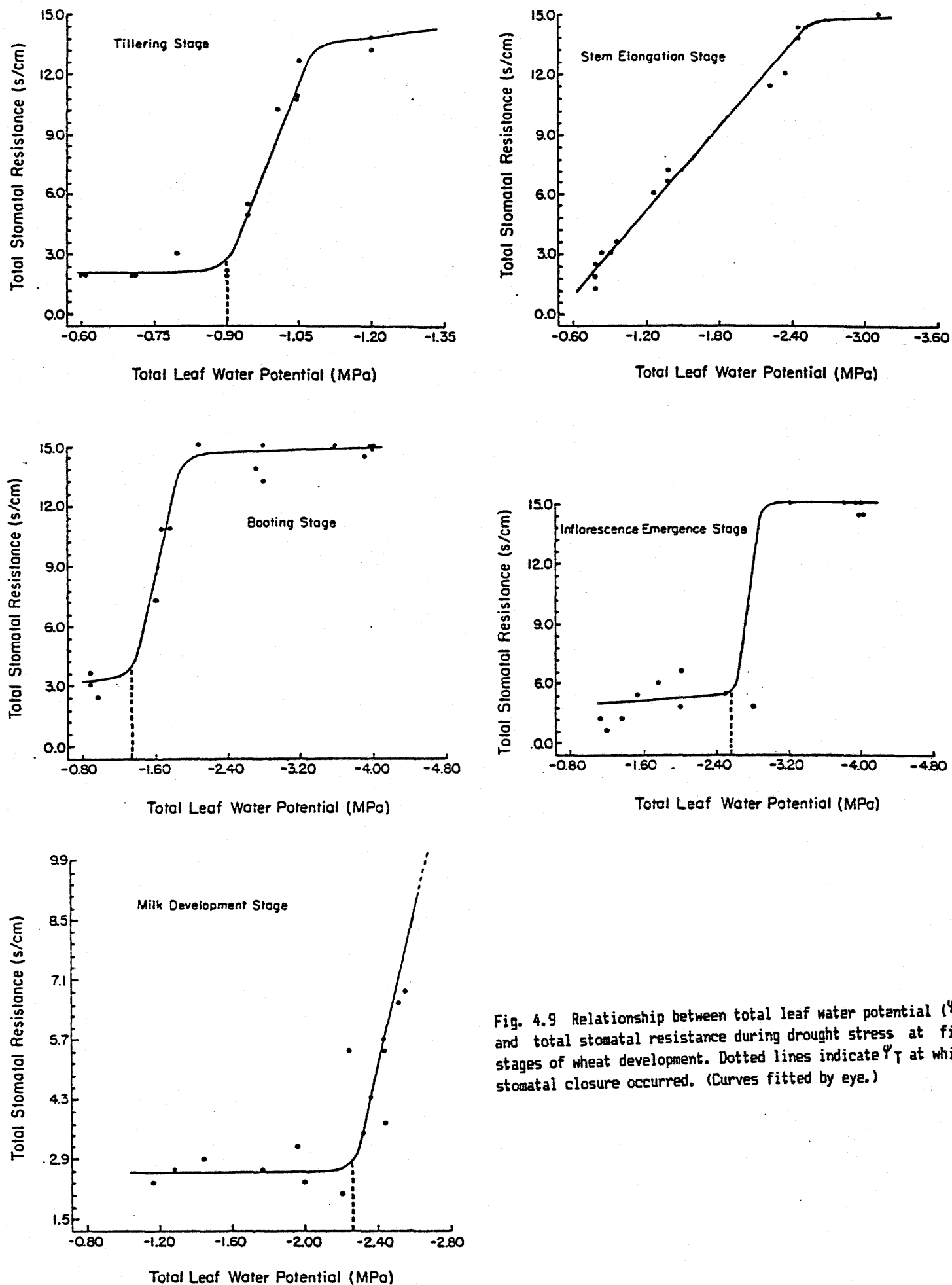


Fig. 4.9 Relationship between total leaf water potential (ψ_T) and total stomatal resistance during drought stress at five stages of wheat development. Dotted lines indicate ψ_T at which stomatal closure occurred. (Curves fitted by eye.)

Table 4.10 Total leaf water potentials (ψ_T) for induction of stomatal resistance and AbA production during drought stress at five stages of wheat development.

Stage of development	Critical ψ_T (MPa) to induce stomatal resistance	Critical ψ_T (MPa) to induce AbA production
Tillering	-0.90	-0.92
Stem elongation	*	*
Booting	-1.35	-1.60
Inflorescence emergence	-2.55	*
Milk development	-2.25	-1.75

* not detectable

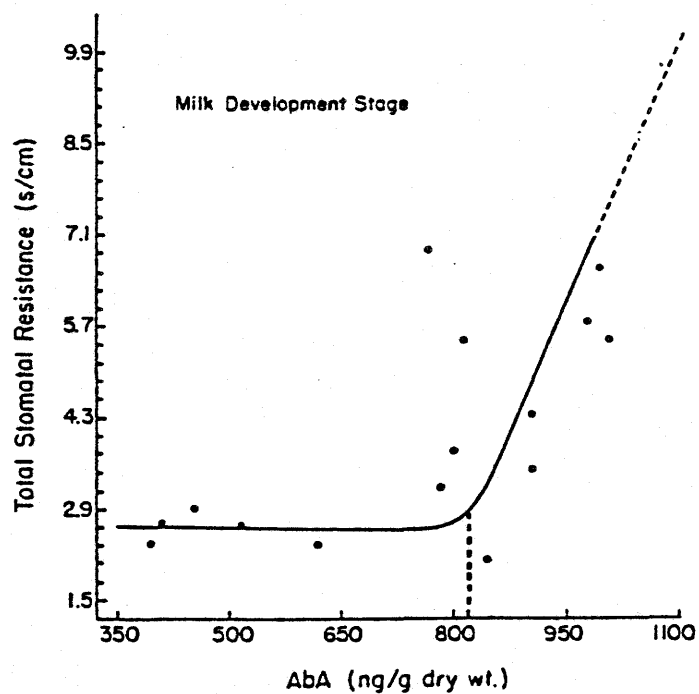
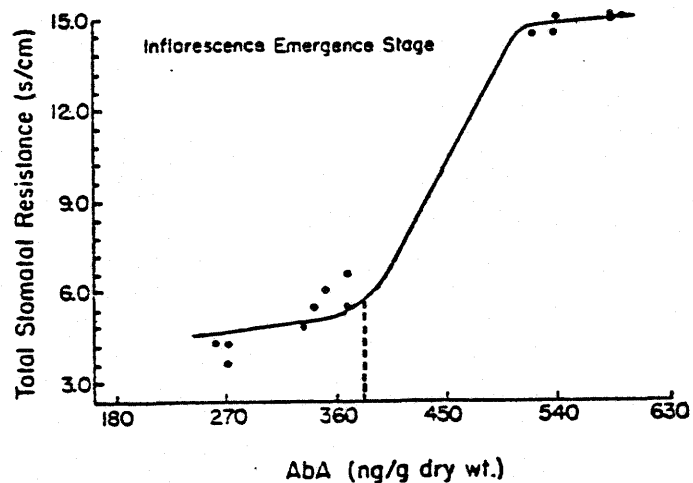
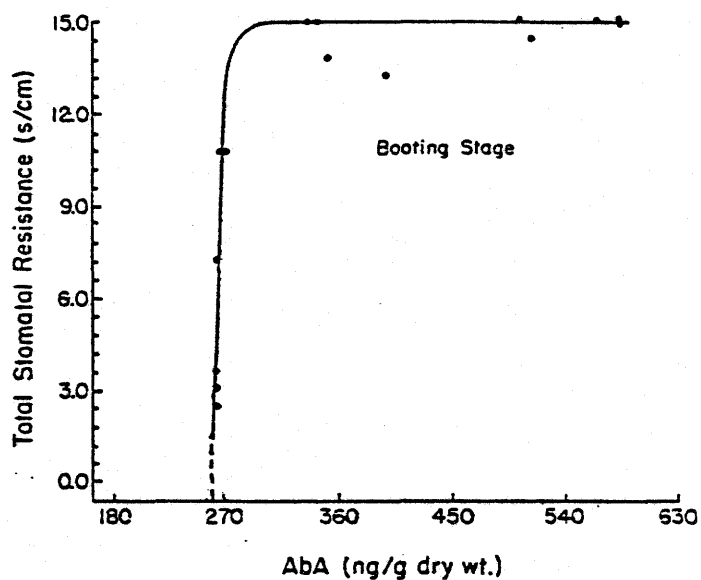
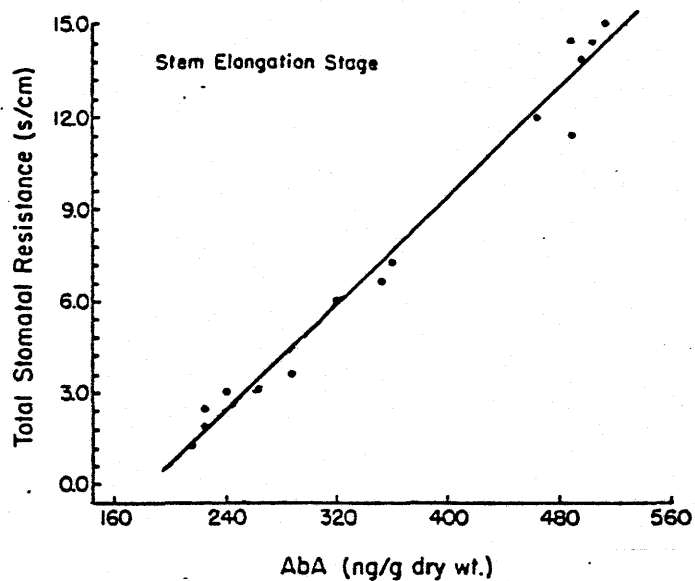
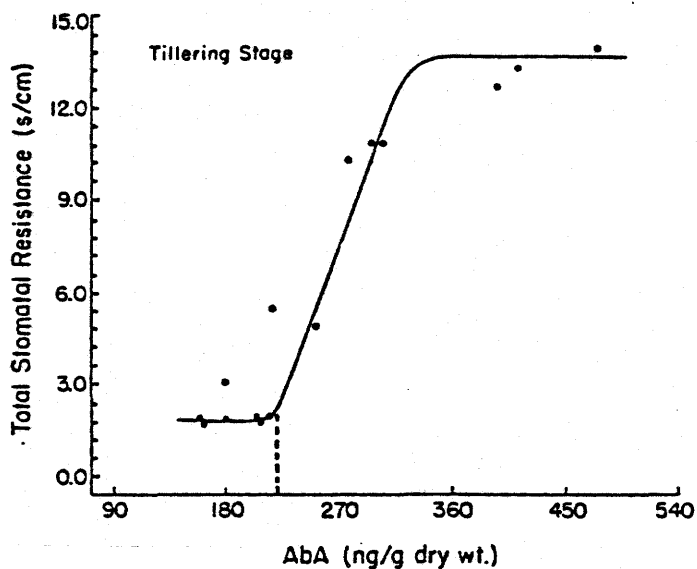


Fig. 4.10 Effect of AbA levels on total stomatal resistance during drought stress at five stages of wheat development. Dotted lines indicate AbA levels at which stomatal closure occurred. (Curves fitted by eye.)

Table 4.11 AbA thresholds for stomatal closure during drought resistance at five stages of development

Stage of development	Critical AbA level for inducing stomatal closure (ng/g dry wt.)
Tillering	217
Stem elongation	*
Booting	256
Inflorescence emergence	378
Milk development	823

* not detectable

however, had a lower (more negative) critical ψ_T than the milk development stage (Table 4.10).

AbA thresholds at which stomatal closure is induced during stress can also be detected (Fig. 4.10 ; Table 4.11). AbA threshold levels follow a similar trend as critical ψ_T for stomatal closure during plant development. Stomatal closure is induced at higher AbA thresholds as the plant grows. Critical ψ_T s for AbA production were generally higher (less negative) than that for stomatal closure (Table 4.10), indicating that there is an increase in AbA production before stomatal closure.

The stage at which total stomatal closure is most sensitive to increases in AbA levels was the booting stage while the least sensitive was the milk development stage (Fig. 4.10; Table 4.11). At the milk development stage also, the amount of AbA required to induce total stomatal closure was more than twice as much as that of any other stage.

4.5.2 Effects of endogenous AbA on the dry matter accumulation of the above-ground parts of the plants

The response of dry matter accumulation in the above-ground parts of the plants to increases in AbA levels is shown in Table 4.12. With the exception of the milk development stage, dry matter accumulation over the stress period increased with the stage of development. The greatest

accumulation of dry matter was at the inflorescence emergence stage while the least accumulation was at the milk development stage. The increase in dry matter content per ng increase in AbA also increased with the stage of development, with the exception of the milk development stage.

Relative growth rates during drought stress, however, steadily declined as the plant grew. The highest relative growth rate was observed at the tillering stage, where the rate of growth was approximately ten times as fast as that of any other stage of development.

4.6 Comparison of AbA sensitivity during plant development

The responses which AbA elicits during plant water stress, viz. increases in stomatal resistance and in dry matter of shoots, and the effects of changes in plant water status on AbA production were compared for all five stages studied. A summary of these comparisons is given in Table 4.13.

Table 4.12 Change in dry matter content of total above-ground parts per ng. increase in ABA level during drought stress at five stages of wheat development.

Stage of development	Dry matter content of shoots (g.)			Increase in ABA production (ng/g dry wt.)	Increase in dry matter, mg/ng increase in ABA level	Relative growth rate (g/g)
	at the onset of stress	at the end of stress period	increase over stress period			
Tillering	0.88	0.27	0.19	251	0.73	2.33
Stem elongation	1.15	1.44	0.29	282	1.83	0.25
Booting	1.95	2.36	0.40	320	1.25	0.21
Inflorescence emergence	4.30	4.95	0.65	320	2.83	0.15
Milk development	8.34	8.66	0.32	582	0.64	0.04

Table 4.13 Summary of AbA effects

Stage of development	Amount of AbA produced during stress (ng/g dry wt.)	Thresholds for AbA production		Critical AbA level for stomatal closure (ng/g dry wt.)	Increase in dry matter, mg/ng increase in AbA
		ψ_T (MPa)	ψ_P (MPa)		
Tillering	260	-0.92	0.13	217	0.73
Stem elongation	282	*	*	*	1.03
Booting	320	-1.60	-0.25	256	1.25
Inflorescence emergence	320	*	*	378	2.03
Milk development	502	-1.75	0.11	823	0.64

* not detectable

5. DISCUSSION

5.1 Changes in AbA levels during water stress and their implications in drought resistance selection

Change in AbA levels during drought stress has now been established as a physiological response in plants. This has been clearly demonstrated again in this study. At each stage of development, there was an increase in the bulk leaf AbA content over the stress period. Changes in absolute AbA were, however, different between the stages of development. This study showed that AbA accumulation during stress increased with age of plants. Exceptionally high AbA levels were obtained at the milk development stage indicating a high degree of sensitivity of AbA production to water stress at this stage. Maximal AbA levels during water stress were also shown to be dependent on age; higher maximal AbA levels were obtained with increasing age of plants. Ilahi and Dorffling (1982) however found contradictory results. In their study on four maize varieties, they found that maximal levels of AbA were higher and were reached earlier in one-month-old than in two or three-month old plants. Nevertheless, they also established that AbA accumulation was age dependent. There is considerable evidence which shows that AbA accumulation during water stress is dependent not only on age, but on the crop species, the degree of stress imposed and on environmental

conditions such as CO₂ concentration and temperature. Beardsell and Cohen (1975) showed that under identical stress conditions AbA increased more in maize than in sorghum plants. Varietal differences in AbA levels during water stress in wheat were shown by Quarrie and Jones, (1979) and Tompkins (1980). Tompkins (1980) showed that of the 14 varieties of wheat field-grown under drought stress conditions, Pitic 62 produced the highest amounts of AbA. The levels obtained by Tompkins (1980) however were much lower than those found in this study.

In trying to determine a stage of development at which varietal differences in AbA accumulation are useful for screening for drought resistance, one would be tempted to look for the stage at which pronounced levels of AbA are observed during water stress. Most reports in which genotypic AbA differences were used in screening for drought resistance fail to justify the stage of development at which the studies were made. Most frequently, genotypic variation is studied in the early vegetative phase, i.e. the seedling growth or tillering stages. Larque-Saavedra and Wain (1976) used the seedling growth stage to detect a positive correlation between drought-induced AbA concentrations and drought resistance in maize and sorghum. Kannangara et al. (1982) confirmed the finding of Larque-Saavedra and Wain (1976) at a post-anthesis stage of growth in sorghum. There are, however, conflicting reports in the case of maize. While Larque-Saavedra established a positive relationship between AbA accumulation and drought resistance in maize at the seedling stage, Ilahi and Dorffling

(1982) showed a negative relative relationship at the same stage of development. Since phasic differences in AbA levels between varieties is dependent on the stage of development, it is important that the stage of growth at which genotypic differences are studied be carefully chosen and be a reflection of its inherent drought-resistant capabilities.

In this study, although there were noticeable changes in AbA levels during drought stress between the various stages of development examined, only very small differences in the relative increase in AbA levels were observed. The constant relative increase in drought-induced AbA throughout the growth of plants may be attributed to the inherent capacity of the cultivar. In a study of genotypic variation in AbA levels in response to drought stress, differences in relative increases in AbA levels among genotypes may be a more important selection index for drought resistance than absolute amounts.

5.2 Sensitivity of AbA to changes in plant water status during wheat development

Thresholds in total leaf water potential and leaf pressure potential for AbA production were established at three stages of development (Figs. 4.7 , 4.8; Table 4.9). Generally the total leaf water potential thresholds at which AbA accumulation was induced were lower as the plant aged, although the stem elongation and inflorescence emergence stages showed slightly higher total leaf water potentials than

the preceding stages. The concept of a water potential threshold was first proposed by Zabadal (1974) in Ambrosia leaves and has since been observed in other crops (Beardsell and Cohen, 1975; Wright, 1977; and Walton et al., 1977). Although Ilahi and Dorffling (1982) could not detect Ψ_T thresholds, they showed that maximal AbA levels in one-month-old stressed maize plants were obtained at higher water potentials than at the older stages. Although measurements of components of leaf water potential, RWC and AbA in this study were done on four different leaves, Etchevers et al. (1982) showed that there were insignificant differences in the water status between the youngest four leaves of the main wheat tiller over the stress period.

Pressure potential thresholds for AbA production were in the range of -0.25 to 0.13 MPa (Table 4.9). This supports the notion that zero or low turgor is the trigger for AbA synthesis (Pierce and Raschke, 1980;1981). Pierce and Raschke (1980) suggested that as the plant grows, changes in cell elasticity, volume and solute content affect values of total leaf water potential and osmotic potential at turgor potentials of zero. It can be observed from the results of this study, that AbA accumulates at different total leaf water potentials at different stages of development according to the total leaf water potential at which turgor becomes zero. This finding may have implications in the mode of action of AbA (section 2.5). Quarrie and Lister (1984) suggested that a trigger factor in the plasmalemma and/or cell wall in response to stress, switches on AbA synthesis in the cytosol. The loss

in turgor, therefore, may bring about these conformational changes in the plasmalemma and/or cell wall that would activate the factor.

5.3 Effects of water stress on plant water status

Frank et al. (1973) showed that ψ_T dropped more rapidly during water stress at the older stages of development. This study showed that ψ_T dropped more rapidly in the stem elongation, booting and inflorescence stages than in the milk development stage. The discrepancy in the findings may be attributed to the different rates of drying of soil at the various stages of development (Fig. 4.2a). It would be expected that the milk development stage would show the greatest depletion of soil water because of greater foliage for transpiration. The slow depletion may be attributed to effects of shading on the lower leaves which increases senescence of lower leaves and hence increases their stomatal resistance.

Morgan (1980) showed that osmotic responses due to changes in ψ_T may be attributed to either concentration effects as a result of loss of water from the cell volume or to osmotic adjustments in which solutes, largely by-products of photosynthesis, soluble sugars, organic and amino acids, and K^+ and Cl^- ions (Turner et al., 1978) are actively taken into the cells. This study showed that there were osmotic adjustments in the booting, inflorescence emergence and milk

development stages (Fig. 4.5). The tillering and stem elongation stages showed no clear evidence of osmotic adjustments. The lack of osmotic adjustments make these stages more sensitive to water stress than the booting, inflorescence emergence or milk development stages. Sionit et al. (1980) found less change in Ψ_{π} relative to RWC during early anthesis than during tillering or dough stages, indicating that plants in the former stage are more susceptible to water stress.

In all the stages studied, osmotic potentials declined less rapidly than total leaf water potentials during drought stress periods resulting in negative values of pressure potential in highly stressed plants. The negative values in turgor potentials, however, were probably an artifact of the technique used in this study. Turgor potentials at the end of stress at the stem elongation, booting and inflorescence emergence stages were lower than that at the tillering or milk development stages.

Osmotic adjustment to mild and slowly developing water stress has been observed in wheat and on other plant species grown under field or greenhouse conditions (Jones and Turner, 1978; Turner et al., 1978). Jones and Turner (1978) observed osmotic adjustments in slowly stressed greenhouse-grown wheat plants only during later stages of growth. In this study, stress developed much more rapidly in the stem elongation, booting and inflorescence emergence stages than in the tillering or milk development stages. In contrast to the findings of Jones and Turner (1978), and Turner et al. (1978),

the tillering stage did not show osmotic adjustments even though stress applied at this stage was more gradual than in the booting, stem elongation or inflorescence emergence stages. Perhaps ψ_T had not declined sufficiently to induce osmotic adjustments. The milk development stage, however, showed osmotic adjustments. Sionit et al. (1980) showed that osmotic adjustments also occur in wheat plants when stressed very rapidly during the early tillering and dough stages but not at the early anthesis stage. In this study, regardless of the intensity of stress, only the booting, inflorescence emergence and milk development stages showed clear osmotic adjustments.

5.4 Effects of endogenous AbA on stomatal resistance

In each stage of development, except the booting stage, the critical ψ_T for AbA accumulation was lower than for stomatal closure (Table 4.10). This suggests that AbA accumulation precedes stomatal closure during water stress. These findings are consistent with numerous reports that show that stomatal closure during water stress is induced by enhanced levels of AbA (Wright, 1972; Jones and Mansfield, 1970; and, Hiron and Wright, 1973). Henson (1982) also showed in a study of sequential responses to water stress in rice leaves, that first AbA levels increase then stomata close and finally leaves roll. However, an adequate understanding of mechanisms cannot be achieved merely by considering bulk leaf AbA in relation to stomatal resistance. Beardsell and Cohen

(1975) showed that an increase in stomatal resistance preceded a marked increase in AbA content in maize plants. Similar findings have been reported in other crop species (section 2.4). The concept of compartmentalization of AbA may explain the "anomaly" in stomatal behaviour to AbA levels (section 2.4).

Another observation made in this study is that there is apparently a time lag between AbA production and induction of stomatal closure at the milk development stage (Table 4.10). A possible explanation for this is that AbA may be imported to the leaves via the xylem as was observed by Loveys and During (1984) in field-grown vine leaves prior to de novo synthesis in the leaves. Another possible reason is that the guard cells may have adapted to existing AbA levels and, therefore, a greater amount of AbA would be needed to induce stomatal closure. These discrepancies in the timing of events call for a more careful study of the amount and location of AbA synthesis in the leaf during stress instead of considering bulk leaf AbA per se.

The relationship between total leaf water potential and total stomatal resistance shows that the critical ψ_T at which total stomatal closure is induced is lower as the plant develops. This confirms the relation between total leaf water potential, stomatal resistance and stage of physiological development as reported by Frank et al. (1973). Teare et al. (1982) working on wheat plants water-stressed at different stages of development also found that the older the plant, the

lower the critical leaf water potential for stomatal closure. They suggest that allowing the closure of stomata during the vegetative stages at high total leaf water potential not only allows for conservation of water until anthesis, but also ensures stomatal opening from anthesis through ear-filling so that optimum gas exchange may occur at a lower total water potential when availability of water to plants is restricted. There is, therefore, a tendency for wheat plants to get more drought resistant at older physiological stages.

5.5 Effects of drought-induced AbA on dry matter accumulation in the above-ground parts of the plant

The results from this study suggest that the developmental stage is a factor which determines the sensitivity of dry matter accumulation to AbA levels. Generally there is an increase in sensitivity of dry matter accumulation in the shoots to AbA during drought stress as the plant matures, i.e. dry matter accumulation increases per ng increase in AbA as the plant grows (Table 4.12). The milk development stage, however, showed lower dry matter accumulation per ng increase in AbA than the other stages indicating a low sensitivity to AbA. Interestingly enough, the milk development stage also had exceptionally high levels of AbA during drought stress indicating that at these levels AbA may have had very inhibitory effects on dry matter accumulation.

It is not possible to determine the extent of AbA involvement on the relative growth rates as relative growth rates themselves generally decrease as the plant matures. Exogenously applied AbA on wheat plants would be necessary to detect AbA effects on the relative growth rates at various stages of development.

6. SUMMARY AND CONCLUSIONS

The effect of physiological age on the accumulation of AbA during drought stress indicates that the degree of AbA accumulation is age dependent. It has been demonstrated that the amount of AbA accumulation increases as the plant matures and that the highest levels of AbA accumulation are observed at the milk development stage.

The milk development stage showed the greatest changes in AbA levels despite relatively low stress during the drought period. There is also a tendency for the plants to withstand stress at older stages of development. The following conclusions may be made on the basis of plant water relations:

i) there is a threshold level in the Ψ_T and Ψ_P at which AbA accumulation is induced,

ii) the Ψ_T threshold for AbA accumulation decreases as the plant grows indicating that drought resistance increases with plant age,

iii) AbA accumulation is triggered off when zero or low turgor is reached regardless of the stage of development,

iv) Ψ_T thresholds for stomatal closure are lower than AbA accumulation indicating that AbA accumulation precedes stomatal closure and that this may be necessary for inducing it,

v) the amount of AbA produced during drought stress increases with the age of plants, making older stages more

sensitive in AbA production to drought stress. Relative increases in AbA levels, however, were the same at all stages of growth.

vi) There was an increase in the dry matter accumulation of the shoots per ng increase in AbA during drought stress as the plant grew. The milk development stage however, showed lower and, consequently, the least sensitivity in dry matter accumulation in response to increases in AbA levels. Relative growth rates during drought stress decreased as the plant matured.

An ideal stage of development at which varietal differences in AbA accumulation during water stress can be used as a selection tool for drought resistance would be the milk development stage. But this is confounded with the finding that drought sensitivity decreases as the plant develops. The milk development stage is more drought tolerant than the younger stages of development. Ideally, a suitable stage would be that in which drought stress would have an effect on both plant water status and AbA accumulation. In this study, the greatest sensitivity in plant water status was observed at the tillering stage while that of AbA accumulation was at the milk development stage. It can be postulated here that if this behaviour is a reflection of other wheat varieties, then selections for AbA accumulation and drought resistance may best be done at an intermediate stage of plant development.

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