Endophytes modulate drought tolerance in pulses by scavenging reactive oxygen species in plant cells under drought stress

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IINTRODUCTION

Abiotic stresses cause major crop loss globally. It is difficult to control the effect of abiotic stresses due to continuous change in climatic conditions. Abiotic stress in fact is the principal cause of crop failure worldwide, reducing major crops yield by more than 50% (Rodziewicz *et al*. 2014). Among abiotic stresses drought is major issue which heavily impact the cool season legumes crop (chickpea, Pea) (Malhotra *et al.* 2004, Subbarao *et al.* 1995).

*Fig.1 Pulses

About 795 million people were estimated to be chronically undernourished in 2014-16 (FAO, 2015). The state of food insecurity in the world makes the high-nutrient pulse varieties (pea, chickpea and lentil) most desirable crop. Pulses are good source of protein, carbohydrate and minerals and also have been found to reduce cholesterol level and the chances of heart diseases. Due to importance of pulses UN General Assembly (68th) announced the 2016 the International Year of Pulses (IYP) (A/ RES/68/231) (FAO, 2016).

Chickpea and pea are major pulse crops which are grown widely but their production is limited due to wide spread drought conditions. Under stress conditions reactive oxygen species (ROS) production leads to oxidative damage (H_2O_2, $^{\cdot}OH$) which causes less plant growth and yield. In many studies it has been found that gene expression for antioxidant enzyme is upregulated to protect plant from cellular injury caused by ROS in response to stress.

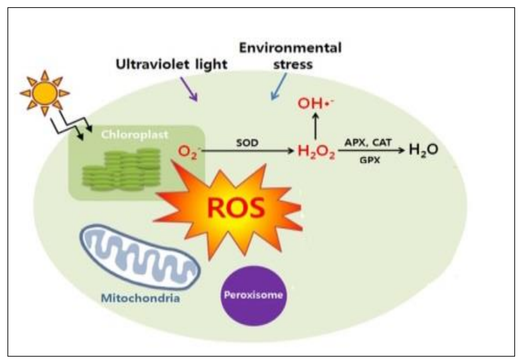

**Fig.2 Overview of ROS production in plant cell

Many studies confirms that fungal endophytes are ROS scavengers but the mechanism by which endophytes may confer tolerance to plants in an extreme environment is still poorly known. Our previous work demonstrated that the endophytes tested in this study have capacity to enhance wheat tolerance against heat and drought stress in both parental plants and second generation seeds via mycovitality (Hubbard *et al*. 2013).

So this study aims to improve pulse crop production by the help of endophytic symbionts that prevents ROS production in plant cells when exposed to abiotic stress.

OBJECTIVES

- To assess effect of endophytes on seed germination efficacy.
- To find out whether the endophytes can scavenge ROS in pulses when exposed to (1) an increased osmotic pressure (IOP) in PDA growth. medium and/or (2) decreased water availability in PEG medium
- Role of endophytes in modulating antioxidant gene expression under drought conditions.

MATERIALS AND METHODS

Seed Germination

Second generation seeds produced from F1 (E- and E+) plants subjected to drought stress were used in this study. Seeds approved free from endophytes were germinated on IOP-PDA and 5% polyethylene glycol (PEG) media, and germination rate recorded.

ROS Detection

- Nikon C2 Confocal laser scanning microscope was used to study ROS in 6 day old roots.
- Excised the same length root from all samples and treated equally and processed quickly under microscope to avoid the photo bleaching. Measured the florescence in form of florescence intensity.
- Effect of drought on plants assessed in form of antioxidant gene expression by qRT-PCR

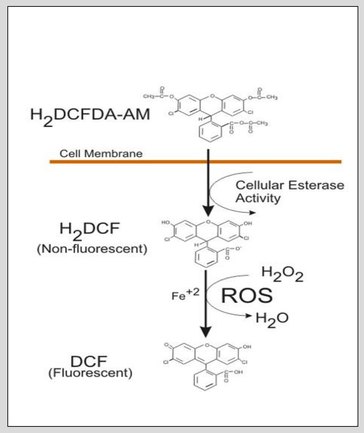

***Fig.3 Formation of fluorescent compound DCF by ROS

RESULTS AND DISCUSSION

Endophytes help in reduction of oxidative damage

ROS production in chickpea and pea increased under both IOP and drought conditions. It was detected by confocal microscopy that there is high green-florescence intensity values in stressed roots.

It was also confirmed that endophyte treatment helps in reduction of ROS production and accumulation in pulses cells. In E+ treatments the fluorescence intensity values were low compared to E- treatments (control).

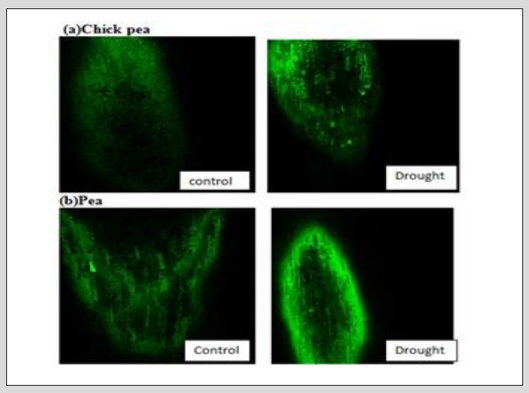

Fig.4 Production of reactive oxygen species under drought conditions in (a) Chickpea and (b) Pea.

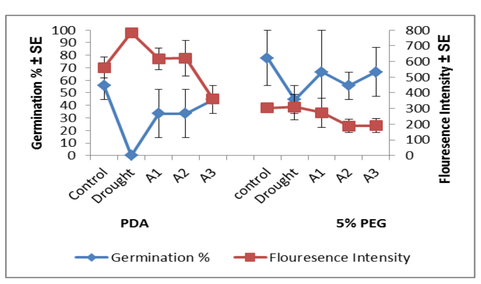

Fig.5 Relation of germination percentage (3rd day) and fluorescence intensity in chickpea on IOP-PDA and 5% PEG. Endophytes treatment (E+) are A1, A2 and A3.

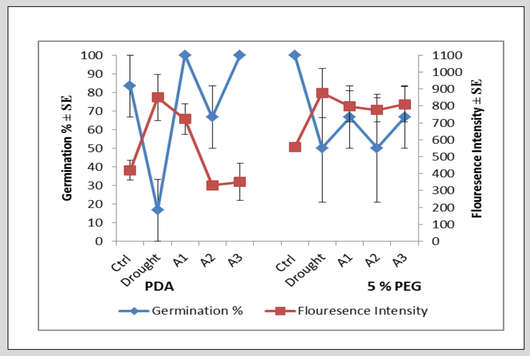

Fig.6 Relation of germination percentage (4th day) and fluorescence intensity in pea on IOP-PDA and 5% PEG. Endophytes treatment (E+) are A1, A2 and A3

Chickpea non-inoculated (E-) samples show higher ROS production under drought , while inoculated (E+) samples exposed to drought are having ROS equal or less than control (Fig.5).

Pea E- and E+ plants show similar pattern in ROS production (Fig.6) to Chickpea plants under drought that might suggest the existence of unique mechanism of symbiotic plant tolerance among pulse varieties via mycovitalism.

Endophyte and seed germination

The use of endophytic symbionts is a promising method by which seed germination can be enhanced (Vujanovic *et al.* 2000). 2nd generation chickpea seed from E- mother plants showed low germination under drought, whereas seed from E+ mother plants increases germination for 20% under drought-stressed conditions (Fig.5). In Pea endophyte A1 and A3 shows more prominent effect on germination than endophyte A2 on IOP-PDA and 5% PEG (Fig.6).

Gene Expression

Under stress conditions antioxidant genes get upregulated to protect plant. But E+ plants show down-regulation of antioxidant genes expressions under both IOP and drought. It seems that endophytes can reduce ROS accumulation and cell oxidative damage by some other pathway yet to be discovered.

CONCLUSION

- Mycovitality increases seed germination % in E+ compared to E- plants under both IOP and drought stresses.
- ROS level measured by fluorescence intensity is inversely related to the germination efficacy.
- Amount of florescence signals produced is directly proportional to amount of ROS present in sample.
- Under drought condition there is an increase in ROS production in E- plant experiencing cell apoptosis.
- Antioxidant genes get up-regulated in E- plants under stress conditions, whereas E+ plants showed down-regulation of these genes for which the mechanism of action is still largely unknown.

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* Fig. 1 http://www.bestcurrentaffairs.com/pulses-inflation-in-india/
** Fig. 2 http://www.frontiersin.org/files/Articles/57335/fpls-04- 00277-HTML/image_m/fpls-04-00277-g001.jpg edited.
***Fig.3 http://www.biotek.com/assets/tech_resources/10592/fig8.jpg

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