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PROLINE ACCUMULATION FOR IMPROVING STAY GREEN TRAIT TO COMBAT WITH ENVIRONMENTAL ABIOTC STRESS IN WHEAT (*TRITICUM AESTIVUM*)

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ABSTRACT

Proline accumulation is an important mechanism for osmotic regulation under abiotic stress. In this study, we evaluated proline accumulation in wheat (*Triticumaestivum*) varietiesafter treatment with Ethyl Methanesulphonate (EMS) of two concentrations (0.5% and 1.0%) for 90minutes. The proline content was observed at booting and maturity stage. At booting stage, proline content was varied from 1.52µmol/gfw in K 7410 to 1.05µmol/gfw in variety HD 2177 in control plants. Variety K 7410 showed highest proline content ranges from 1.52 to 1.65µmol/gfw after the two treatments of EMS. At maturity stage, the proline content varied from 1.09 to 2.22µmol/gfw in control plants, 1.04 to 2.35µmol/gfw after treatment one (0.5%) and 1.00 to 2.46µmol/gfw after treatment second(1%) of EMS. Whereas, variety HD 2177 and K68 shows significant decreased level of proline content after two treatments *i.e.* 1.27 to 1.08µmol/gfw and 1.48 to 1.01µmol/gfw. On the whole, these ten wheat varieties showed decreased level of proline after two treatments of EMS except K 7410 at both the stages. Such results indicates that EMS mutagen had changed some genetic alteration which led to high proline accumulation in wheat varieties which may perform better under abiotic stress conditions such as drought, salt, high temperature etc.

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INTRODUCTION

Growth and productivity of major cereal crops like wheat, rice, maize etc. is greatly affected by environmental stresses such as drought, high salinity, and low temperature due to their sessile nature (Boyer, 1982; Eriksson *et al.*, 2011).Abiotic stress is also known as Osmotic stress which include stomatal closure decrease in photosynthesis and growth inhibition, accumulation of proline, mannitol etc. (Nagar *et al.*, 2015). So, to survive in such stressed environment, plants have developed a number of physiological and biochemical mechanisms (Bartels and Salamini, 2001; Wang *et al.,* 2003). These mechanisms initiate the expression of a variety of genes against the abiotic stresses by plants.Such genes help in protecting cells from stress by producing important metabolic proteins such as key enzymes for osmolytes biosynthesis mainly proline, water channel proteins, sugar and proline transporters, chlorophyll a/b binding protiens(Fowler *et al.,* 2002; Kreps *et al.,* 2002; Seki *et al.,* 2002). Stay-green character, has been identified as an important component in the genetic improvement of several crops to promote stress tolerance and yield gain (Peigao *et al.,* 2006). Stay green phenotypes maintain green leaf area (chlorophyll) for a longer period(photosynthesis), delay leaf senescencewhich help the plant to perform better in abiotic stress conditions(Spano*et al.*, 2003;Luche*et al.*, 2015). Crops grown in stress environments as its yield is associated with the capacity of the plant to maintain CO_2 assimilation (Hafsi*et al.,* 2007).Thus,this vital trait should be incorporated by developing genes for it using mutagens etc. into major crops like wheat, rice etc. Thomas and Howarth (2000) described five types of stay-green phenotypes: Type A phenotypes shown late initiation of senescence with a normal senescence rate. Type B phenotypes show normal initiation of senescence with a slower rate of senescence. Type C phenotype shows lesion in chlorophyll degradation, leaving the rest of the senescence process unaffected. Type D phenotype shows rapid death (freeze, boil, dry) ensures maintenance of leaf colour in dead leaf. Type E: enhanced greenness but unchanged initiation and rate of senescence. As a result the overall process of senescence will take longer to complete. Types A, B and possibly E are functionally stay-green: they maintain photosynthetic capacity in their green tissues. Therefore, they may be a potential means to improve grain yield. In China wheat lines with a wheat-rye chromosome translocation were developed which showed a functional stay-green phenotype combined with increased grain yield and total biomass of up to 25% when grown in the field (Chen *et al.*, 2010; Luo *et al.*, 2006). Under abiotic stress conditions not onlythe stay green trait will help but also the accumulation of important metabolic ptrotiensknown as "compatible osmolytes" mainly proline, will help the plant to overcome the abiotic stress.The stay-green trait has been reported to increase yields (Gong *et al.,* 2005; Christopher *et al.,* 2008; Chen *et al.,* 2010), and there were positive correlations to water use efficiency, nitrogen use, leaf senescence with chlorophyll content(G´orny and Garczy´ nski, 2002; Kipp *et al.,* 2013; Gwendolin *et al.,* 2015), yields under heat and drought (Naruoka *et al.,* 2012).Many'stay green' mutants, referred to as functional stay green or non-yellowing in various plant species have been reported to maintain leaf greenness after the grain-ripening stage and give better yield (Spano *et al.*, 2003; Walulu *et al.,*1994). In wheat, the maintenance of proteins embedded in the walls of thylakoids and low levels of reactive oxygen species demonstrated the superiority of wheat mutant *tasg1* tolerant to drought (Tian*et al.*, 2013). The stress tolerance varieties of wheat screened by breeding method (Arya *et al.*, 2013)and may be studied at moleclar level for stay green trait and proline. Therefore, the present research work has aimed to bring light to major aspects of the stay-green trait and proline for high biomass yield of crops as they both collectively play vital role in maintaining chlorophyll content, photosynthesis duration and osmotic potential under stressed condition.

MATERIALS AND METHODS

The present research work was carried out at laboratory of Department of Biotechnology, College of Agriculture, of S.V.P. University of Agriculture and Technology, Meerut during rabi season. A total of ten varieties of wheat(*Triticumaestivum*) cultivars viz. HUW 510, C 306, Sonalika, HD 2135, HD 2177, VL 401, K 9162, RAJ 3765, K 68, K 7410 were collected to study the effect of EMS treatment on biochemical character i.e. proline content. The seeds of all ten varieties were treated with EMS (0.5% and 1% in distill water) for 90 minutes in petri plates with three replicates. Thereafter, the treated seeds of wheat were sown in pots and the proline content is observed from three replicates two times one at booting stage and second at final maturity stage of control and treatments.

Biochemical Characterization

Proline determination proceeded according to Bates *et al.,* 1973 based on proline's reaction with ninhydrin. For estimation of Proline content in wheat germplasm, 100 mg of fresh leaf tissue was taken from plants. Grind the leaf tissue in aqueous sulphosalisylic acid (3%). Centrifuged the tubes at 7000 rpm for 5 min. The supernatant was mixed with equal volume of Glacial acetic acid. Then add 0.5 ml of ninhydrin and incubated for 30 minute at 100 in boiling water bath and then placed for 5 min in ice bath for cooling. Thereafter 2 ml of toluene was mixed in reaction mixture and mixed properly. The aqueous phase was transferred in new tube. The reaction mixture was warm at 25^{0}C and chromophore was measured at 520nm was determined in a BioMate spectrophotometer (ThermoSpectronic).

Statistical analysis

The experimental data obtained from randomly selected five plant from each replicates were subjected to the statistical analysis outlined by Panse and Sukhatme (1978). The significance of differences among treatment means was tested by 'F' test and critical differentiation (at 5 per cent probability)was calculated by the method given by Pearson K., 1895.

RESULTS AND DISCUSSION

Proline is an important parameter to know the tolerance capacity of the plants in stress coditions(Delauney and Verma, 1993), and its accumulation indicates the response against abiotic stresses like drought, salt, high temperature etc. (Ramanjulu andSudhakar, 2000).Among the studied wheat(*Triticumaestivum*)varieties the proline content at booting stage in the leaf varied from 1.52μmol/gfw in K 7410 to 1.05 μmol/gfw in variety HD 2177 in control plants (Table 1). All the variety showed a little decrement in the amount of total proline after the treatments of EMS except in Sonalika, K 7410 and RAJ 3765(Figure 1). The highest proline content was shown by variety K 7410 and it found to be increases from 1.52 to 1.65μmol/gfw after the two treatments of EMS. Variety Sonalika and RAJ 3765 also shownincrement in proline content from 1.21 to 1.29μmol/gfw and 1.49 to 1.55μmol/gfw. Whereas, the variety HD 2177 showed the lowest amount of proline content and found to be decreases from 1.05 to 0.99mol/gfw after all two treatments. The similar pattern of increment of proline content was also observed at maturity stage in present investigation. The proline content varied from 1.09 to 2.22μmol/gfw in control plants(Table 2). The amount of total proline content after the treatments of EMS in Sonalika, K 7410 and RAJ 3765 shows an increment level among from all wheat varieties. The wheat varieties other than Sonalika, K 7410 and RAJ 3765 shows slight decrease in proline level after two treatments but variety HD 2177 and K68 shows significant decreased level of proline content after all treatments 1.27 to 1.08μmol/gfw and 1.48 to 1.01μmol/gfw(Figure 2). The highest proline accumulation was shown by variety K 7410 after all treatments of EMS from 2.22 to 2.46μmol/gfw. High proline synthesis in plants could favor a better recovery of plants in stressed conditions, converted in grain yield to a

Table 1: Proline content of ten wheat varieties of control and two treatments of EMS (at booting stage)

S.No.	Variety	Proline (μmol/gfw) Control		T1		T2	
		Mean	SE	Mean	SE	Mean	SE
1	HUW 510	1.21	±0.019	1.20	±0.006	1.18	±0.006
2	C 306	1.12	±0.043	1.05	±0.018	1.04	±0.015
3	Sonalika	1.21	±0.012	1.27	±0.025	1.29	±0.019
4	HD 2135	1.30	±0.009	1.28	±0.015	1.25	±0.009
5	HD 2177	1.05	±0.007	1.00	±0.012	1.00	±0.006
6	VL 401	1.40	±0.006	1.38	±0.021	1.33	±0.031
7	K 9162	1.08	±0.012	1.06	±0.012	1.04	±0.012
8	RAJ 3765	1.49	±0.013	1.52	±0.017	1.55	±0.012
9	K 68	1.28	±0.022	1.11	±0.007	1.10	±0.007
10	K 7410	1.52	±0.015	1.57	±0.009	1.65	±0.015
	S.Em±			0.214			
	C.D. (p=0.05)			0.073			

T1= 0.5% EMS, T2= 1.0% EMS

Table 2: Proline content of ten wheat varieties of control and two treatments of EMS (at maturity stage)

S.No.	Variety	Proline (µmol/gfw)					
		Control		T1		T2	
		Mean	SE	Mean	SE	Mean	SE
1	HUW 510	1.51	±0.020	1.55	±0.009	1.53	±0.004
2	C 306	1.32	±0.053	1.25	±0.020	1.20	±0.017
3	Sonalika	1.57	±0.022	1.59	±0.029	1.61	±0.009
4	HD 2135	1.32	±0.019	1.26	±0.019	1.21	±0.009
5	HD 2177	1.27	±0.017	1.11	±0.018	1.08	±0.008
6	VL 401	1.50	±0.016	1.45	±0.027	1.40	±0.038
7	K 9162	1.09	±0.011	1.04	±0.015	1.00	±0.014
8	RAJ 3765	2.09	±0.018	2.19	±0.016	2.25	±0.011
9	K 68	1.48	±0.029	1.11	±0.005	1.01	±0.009
10	K 7410	2.22	±0.012	2.35	±0.010	2.46	±0.011
	S.Em±			0.321			
	C.D. (p=0.05)			0.123			

T1 = 0.5% EMS, T2 = 1.0% EMS

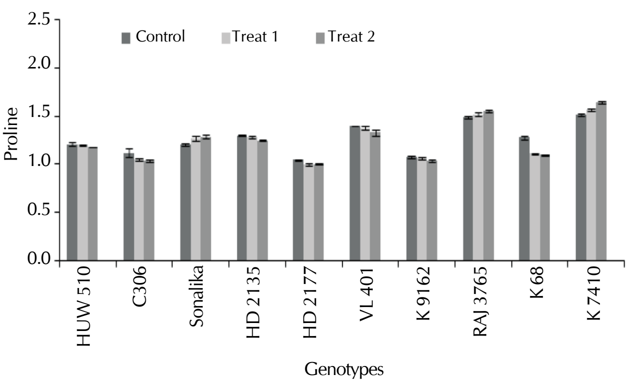

Figure 1: Graph representing the proline content in wheat at booting stage

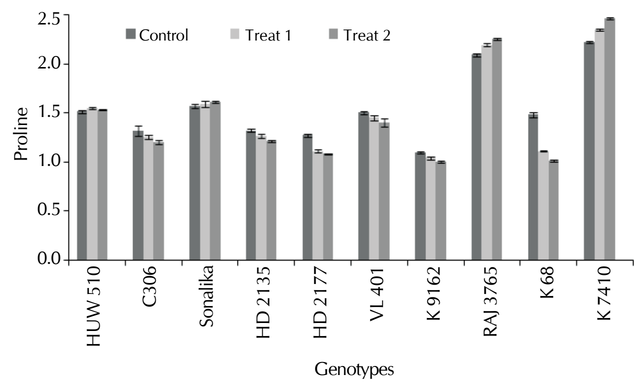

Figure 2: Graph representing the proline content in wheat at maturity stage

protected photosynthetic apparatus after water field status recovery. The similar results of proline levels increase in wheat under stress condition pointed by Nayyar (2003) and Zhu *et al.* (2005). Similarly, results are in agreement with those obtained by Chen and Li (2002), who showed that a high concentration of proline in suspension cells avoided lipid peroxidation (Parvanova *et al.,* 2004). Numerous studies have linked the accumulation of proline to abiotic stress (Aziz *et al.,* 1999; Munns and Tester, 2008; Ribeiro *et al.*, 2014), and it can play a protective role against the osmotic potential, membrane instability generated by salt, heat etc.(Chen *et al.,* 2007; Hoque *et al.,* 2008; Satbhai *et al.,* 2014). Proline accumulation under drought stress has been reported in other crop also like chick pea (Ayerb and Tenori, 1998), corn (Serraj and Sinclair, 2002) and peanut (Smith *et al.,* 2002). Huang *et al.* (2013) had determined the relationship between salt tolerance and proline content in Jerusalem artichoke plantlets. Synthesis or accumulation of proline is depending on the activities of enzymes such as pyrroline-5-carboxylate synthetase (P-5-C synthetase), pyrroline 5-carboxylate reductase etc. Several investigators (Ramanjulu and Sudhakar, 2000; Kumar *et al.,* 2003) reported a positive correlation between the accumulation of proline and its osmoprotective role at the whole plant level during abiotic stress conditions mainly in drought, salt etc. Similarly, in the present study a positive correlation between free proline accumulation and stay green trait in wheat cultivars in control and treated plants which can adopt better themselves in abiotic stress conditions. Stay green trait help in maintaing the long photosynthetic duration and chlorophyll content which results better yield (Christopher *et al.,* 2008; Chen *et al.,* 2010).Total flag leaf photosynthesis, chlorophyll content, the onset of senescence and green leaf duration have all been found to be positively correlated with wheat grain yield (Kichey *et al.*, 2007; Wang *et al.*, 2008; Gaju*et al.*, 2011). Hui *et al.* (2012) reported the generation of wheat mutant *tasgl*, induced using EMS exhibits delay in leaf senescence in term of chlorophyll. Four or five classes of delayed senescence or 'stay-green' have been described in the literature (Thomas and Smart, 1993; Thomas and Howarth, 2000) and the stay-green phenotype has shown proven utility to improve yields under abiotic stress (Harris *et al.*, 2007; Vijayalakshmi *et al.*, 2010) and also under biotic stress, like spot blotch (Joshi *et al.*, 2007). High level of Proline could increase the concentration of cell protoplasm, to maintain normal membrane function at high temperatures to be conducive to plant resistance of environmental stress, result proven in tomatoes byYin *et al.* (2001). The present investigation further supports the stress tolerance of the Sonalika, K 7410 and RAJ 3765 based on greater maintenance of proline content coupled with stay green trait i.e. long duration leaf chlorophyll stability andphotosynthesis aftertreatment of EMS. The study of stay-green character can be benificial in breeding programs, which helps in significant genetic progress for attributes such as high yield, industrial quality, disease resistance and tolerance to abiotic stresses. The understanding of the proline accumulation mechanism associated with leaf senescence and photosynthetic efficiency in several major crops may be the key to delete the plateau of productivity associated with adaptation to unfavorable environmental conditions.

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