International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 7, Issue 14, 2015, pp.-875-878.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

ROLE OF PROLINE AND STAY GREEN TRAIT IN IMPROVING PLANT RESISTANCE AGAINST ABIOTIC STRESS IN WHEAT

SINGH N. P.
Department of Biotechnology, SVP University of Agriculture and Technology, Meerut, 250110, India
*Corresponding Author: Email-naresh.singh55@yahoo.com

Received: October 25, 2015; Revised: December 06, 2015; Accepted: December 07, 2015

Abstract- At present, abiotic stresses is emerging as the problem of great concern for the agriculture ecosystem. Under abiotic stresses plants greatly affected at molecular, physiological and biochemical level. Moreover, stay green phenotypes have the capability to maintain longer green leaf area and also helps in triggering the production of osmoprotectants such as proline etc. in stressed environment, which directly results in good yield. Present research was conducted to observe proline accumulation in wheat varieties after treatment with Ethyl Methanesulphonate (EMS) of three concentrations (0.25%, 0.75% and 1.25%) for 90 minutes. The proline content was observed at booting and maturity stage. At booting stage, proline content was varied from 1.45 µmol/gfw in K 7410 to 1.05 µmol/gfw in variety HD 2177 in control plants. Variety K 7410 showed highest proline content ranges from 1.45 to 1.75 µmol/gfw after the three treatments of EMS. At maturity stage, the proline content varied from 1.05 to 2.12 µmol/gfw in control plants, 1.04 to 2.25 µmol/gfw after treatment one(0.25%), 1.00 to 2.36 µmol/gfw after treatment second(0.75%) and 1.00 to 2.65 µmol/gfw after treatment third(1.25%) of EMS. Whereas, variety HD 2177 and K 68 shows significant decreased level of proline content after three treatments. On the whole, these ten wheat varieties showed decreased level of proline after three treatments of EMS except K 7410 at both the stages. Such results indicates that treatment of EMS help in developing such wheat varieties which may perform better under abiotic stress conditions such as drought, salt etc.

Keywords- Ethyl Metanesulphonate, Stay green, Biochemical, Proline, Wheat, Chlorophyll, Osmolytes

Citation: Singh N. P. (2015) Role of Proline and Stay Green Trait in Improving Plant Resistance Against Abiotic Stress in Wheat. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 7, Issue 14, pp.-875-878.

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 [1,2].To survive in such stressed environment, plants have developed a number of physiological and biochemical mechanisms [3,4]. 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 chaperones, osmotin, antifreeze proteins, key enzymes for osmolyte biosynthesis such as proline, water channel proteins, sugar and proline transporters collectively regulating the genes for signal transduction in the stress response [5-7]. Stay green phenotypes maintain green leaf area(chlorophyll) for a longer period(photosynthesis) which help the plant to perform better in abiotic stress conditions [8,9]. Thus, this vital trait should be incorporated into major crops like wheat, rice etc. grown in stress environments as its yield is associated with the capacity of the plant to maintain CO_2 assimilation [10]. [11] described five types of stay-green phenotypes: Type A phenotype shown late initiation of senescence with a normal senescence rate. Type B phenotype shows normal initiation of senescence with a slower rate of senescence. On the other hand, 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 [12,13]. Under abiotic stress conditions not onlythe stay green trait will help but also the accumulation of important metabolic protiensknown as "compatible osmolytes" mainly proline, will help the plant to overcome the abiotic stress. These compounds are thought to play a pivotal role in plant cytoplasmic osmotic adjustment in response to drought, salt, osmotic stresses [14]. The stay-green trait has been reported to increase yields [12,15] and there were positive correlations to water use efficiency [15-17], yields under heat and drought [18]. Various plant species have been reported to maintain leaf greenness after the grain-ripening stage which are referred to have functional stay green trait and results in better yield [8,19,20].The stress tolerance varieties of wheat screened by breeding method [21] and may be studied at molecular level for stay green trait and proline. Therefore, this work aims to bring light on stay-green trait and proline accumulation for high yield production of crops under stressed environment.

MaterialsandMethods

The present research work was carried out at the laboratory of Department of Biotechnology, College of Agriculture, S.V.P. University of Agriculture and Technology, Meerut (India), during the rabi season. A total of ten varieties of wheat 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 i.e. proline content. The seeds of all ten varieties were treated with EMS (0.25%, 0.75% and 1.25% in distilled water) for 90 minutes in petri plates. Thereafter, the treated seeds of wheat were sown in pots and the proline content was observed from three replicates twice, first at booting stage and second at final maturity stage for all the treatments.

Biochemical Characterization

Proline determination proceeded according to [22] 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 and grinded in aqueous sulphosalicylic acid (3%). The tubes were centrifuges at 7000 rpm for 5 minutes. The supernatant was mixed with equal volume of glacial acetic acid and 0.5 ml of ninhydrin and incubated for 30 minute at 100°C in boiling water bath and then placed for 5 minutes in the 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 proline content in samples were measured at 520 nm using BioMate spectrophotometer (ThermoSpectronic).

Statistical analysis

The experimental data obtained from randomly selected five plants from each replicate were subjected to the statistical analysis outlined by [23]. 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 [24].

Result and Discussion

Proline is an important parameter to know the tolerance capacity of the plants in stress conditions [25], and its accumulation indicates the response against abiotic stresses like drought, salt, high temperature etc. [26]. At the booting stage, proline content in the leaf of wheat varieties varied from 1.45 µmol/gfw in K 7410 to 1.05 µmol/gfw in variety HD 2177 in control plants [Table-1].

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

S.No.	Variety	Proline(µmol/gfw)							
		Control		T1		T2		T3	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	HUW 510	1.18	±0.019	1.20	±0.006	1.18	±0.006	1.12	±0.006
2	C 306	1.10	±0.043	1.05	±0.018	1.04	±0.015	1.00	±0.010
3	Sonalika	1.19	±0.012	1.24	±0.025	1.27	±0.019	1.30	±0.012
4	HD 2135	1.20	±0.009	1.24	±0.015	1.30	±0.009	1.15	±0.022
5	HD 2177	1.05	±0.007	1.00	±0.012	1.00	±0.006	0.99	±0.015
6	VL 401	1.30	±0.006	1.35	±0.021	1.55	±0.031	1.11	±0.049
7	K 9162	1.08	±0.012	1.06	±0.012	1.04	±0.012	1.01	±0.022
8	RAJ 3765	1.29	±0.013	1.32	±0.017	1.40	±0.012	1.48	±0.006
9	K 68	1.15	±0.022	1.11	±0.007	1.07	±0.007	1.02	±0.017
10	K 7410	1.45	±0.015	1.50	±0.009	1.55	±0.015	1.75	±0.003
	SEm±	0.213							
	CD (P=0.05)	0.063							

T1= 0.25%EMS, T2= 0.75%EMS, T3= 1.25%EMS

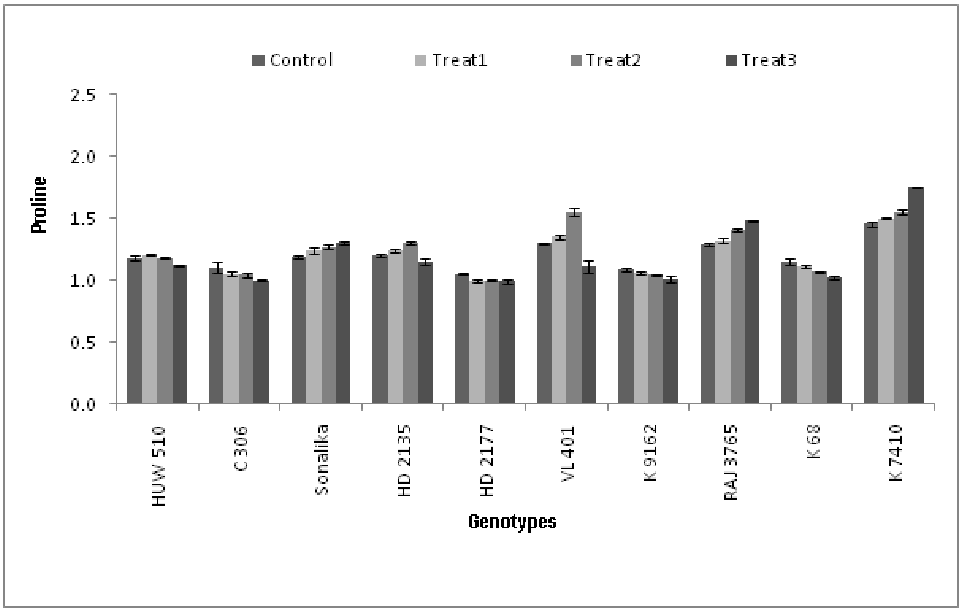

Fig-1 Graph representing the proline content at booting stage in ten wheat varieties.

All the varieties at this stage showed a little decrement in the amount of total proline content after three treatments of EMS [Fig-1]. But K 7410, Sonalika and RAJ 3765 varieties had shown increment in proline content from 1.45 to 1.75 µmol/gfw, 1.19 to 1.30 µmol/gfw and 1.29 to 1.48 µmol/gfw after the treatment of EMS. Furthermore, the variety HD 2177 showed the lowest amount of proline content that decreased from 1.05 to 0.99 mol/gfw after all three treatments. While, at the maturity stage the proline content varied from 1.05 to 2.12 µmol/gfw in control plants [Table-2]. The total proline content in Sonalika, K 7410 and RAJ 3765 have shown an increased level among all the wheat varieties after all three treatments of EMS [Fig-2]. But varieties HD 2177 and K68 showed a significant decrease in proline content after all three treatments i.e. 1.20 to 0.99 µmol/gfw and 1.23 to 1.03 µmol/gfw. Exceptionally, at both stages variety K 7410 showed highest proline content among all wheat varieties after all three treatments of EMS [Table-2]. But varieties HD 2135 and VL 401 exhibited the increment in proline content forfirst two treatments but in third it showed sudden downfall in proline content at both stages [Fig-2]. As high proline synthesis may be helpful in protecting photosynthetic apparatus of plants in stressed environment to give better yield. The similar results of proline levels increase in wheat under stress condition pointed by [27,28]. [29] also showed that a high concentration of proline in suspension cells avoided lipid peroxidation [30].

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

S.No.	Variety	Proline(µmol/gfw)							
		Control		T1		T2		T3	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	HUW 510	1.31	±0.020	1.28	±0.009	1.23	±0.004	1.17	±0.006
2	C 306	1.32	±0.053	1.25	±0.020	1.20	±0.017	1.11	±0.010
3	Sonalika	1.39	±0.022	1.45	±0.029	1.50	±0.009	1.53	±0.012
4	HD 2135	1.26	±0.019	1.29	±0.019	1.35	±0.009	1.13	±0.022
5	HD 2177	1.20	±0.017	1.14	±0.018	1.08	±0.008	0.99	±0.015
6	VL 401	1.40	±0.016	1.44	±0.027	1.48	±0.038	1.21	±0.049
7	K 9162	1.05	±0.011	1.04	±0.015	1.00	±0.014	1.00	±0.022
8	RAJ 3765	2.00	±0.018	2.09	±0.016	2.15	±0.011	2.24	±0.006
9	K 68	1.23	±0.029	1.18	±0.005	1.13	±0.009	1.03	±0.017
10	K 7410	2.12	±0.012	2.25	±0.010	2.36	±0.011	2.65	±0.003
	SEm±	0.303							
	CD (P=0.05)	0.117							

T1= 0.25%EMS, T2= 0.75%EMS, T3= 1.25%EMS

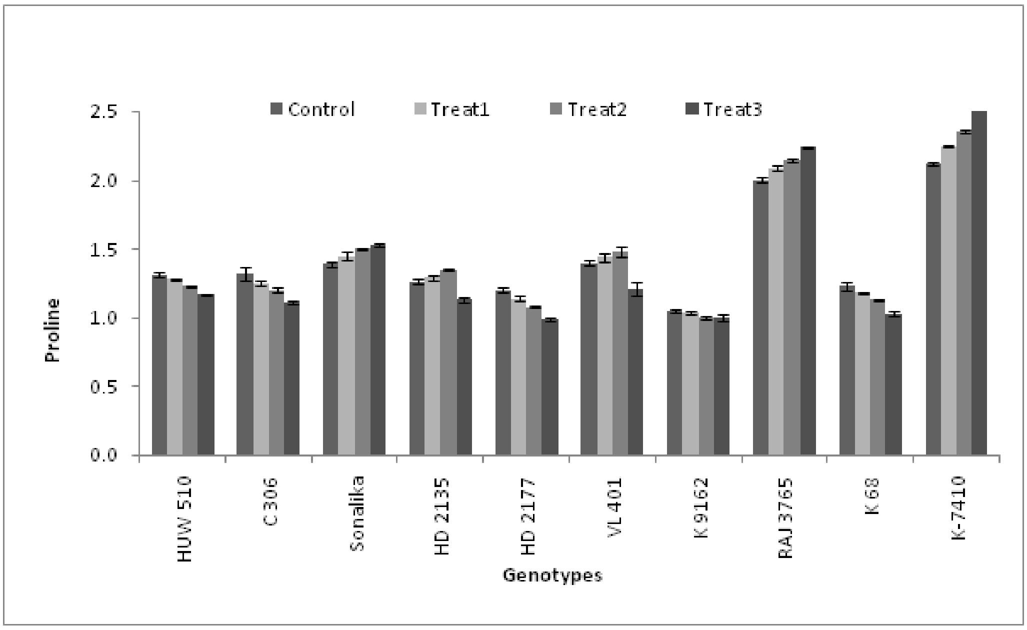

Fig-2 Graph representing the proline content at maturity stage in ten wheat varieties.

Numerous studies have linked the accumulation of proline to abiotic stress [31,32] and it can play a protective role against the osmotic potential generated by salt [33,34]. Proline accumulation under drought stress has been reported in other crop also like chickpea [35], corn [36] and peanut [37]. [38] 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 [26,39] 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.[40]reported the generation of wheat mutant *tasgl*, induced using EMS exhibits delay in leaf senescence in term of chlorophyll.. Similarly, in the present study a positive correlation between free proline accumulation and stay green trait in wheat cultivars in controlled and treated plants, which can adopt themselves better in abiotic stress conditions. Stay green traits help to maintain the long photosynthetic duration and chlorophyll content which results in better yield [12,15]. High level of Proline also protect the plants from high temperature environment [41]. The present investigation further support the abiotic 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 of photosynthesis by maintaining longer leaf chlorophyll content after treatment of EMS. The understanding of the proline accumulation mechanism associated with stay green trait i.e. delayed leaf senescence and longer photosynthetic efficiency in several major crops may be the key to overcome the productivity loss under unfavorable abiotic environmental stresses.

Conflict of Interest: None declared

References

[1] Boyer J. S. (1982) *Science,* 218, 443–448.
[2] Eriksson S.K. and Harryson P. (2011) *Dehydrins: molecular biology, structure and function, Plant Desiccation Tolerance, pp289-305 (edsLutte et al, Springer verlag).*
[3] Bartels D. and Salamini F. (2001) *Plant Physiology,* 127, 1346-1353
[4] Wang W., Vinocur B. and Altman A. (2003) *Planta,* 218, 1–14
[5] Flowers T.J. and Yeo A.R. (1995) *Aust. J. Plant Physiol*., 22, 875–884.
[6] Seki M., Narusaka M., Ishida J., Nanjo T., Fujita M., Oono Y., Kamiya A., Nakajima M., Enju A. and Sakurai T. (2002) *Plant J.,* 31, 279–292.
[7] Nagar S., Aroral A., Singh V.P., Ramakrishnan S., Umesh D.K., Kumar S. and Saini R.P. (2015) *The Bioscan*, 10(1), 67-72.
[8] Spano G., FonzoN. Di., Perrotta C., Platani C., Ronga G., Lawlor D.W., Napier J.A. and Shewry P.R. (2003) *Journal of Experimental Botany,* 54, 386.
[9] Luche H.S., Silva J.A.G., Maia L.C. and Oliveira A.C. (2015) *Santa Maria*, 45(10), 1755-1760.
[10] Hafsi M., Akhter J. and Monneveux P. (2007) *Cereal Research Communications,* 35, 71-80.
[11] Thomas H. and Howarth C.J. (2000) *J. Exp. Bot*., 51, 329-337.
[12] Chen J.B., Liang Y., Hu X.Y., Wang X.X., Tan F.Q., Zhang H.Q., Ren Z.L. and Luo P.G. (2010) *Acta. Physiol. Plant.,* 32, 875-882.
[13] Luo P.G., Ren Z.L., Wu X.H., Zhang H.Y., Zhang H.Q. and Feng J. (2006) *Chin. Sci. Bull.,* 51, 2595 2603.
[14] Wyn Jones R.G., Storey R., Leigh R.A., Ahmad N. and Pollard A. (1977) *Elsevier, Amsterdam,* pp. 121-136.
[15] Christopher J.T., Manschadi A.M., Hammer G.L. and Borrell A.K. (2008) *Australian Journal of Agricultural Research,* 59, 354-364.
[16] G´orny A.G. and Garczy´nski S. (2002) *J. Appl. Genet.,* 43, 145–160.
[17] Kipp S., Bodo M. and Schmidhalter U. (2013) *Functional Plant Biology,* 41(3), 227-235.
[18] Naruoka Y., Sherman J.D., Lanning S.P., Blake N.K., Martin J.M. and Talbert L.E. (2012) *Crop Sci.,* 52, 99–109.
[19] Fang Z., Bouwkamp J.C. andSolomos T. (1998) *J. Exp. Bot*., 49,503–510.
[20] Walulu R.S., Rosenow D.T., Wester D.B. and Nguyen H.T. (1994) *Crop Sci.,* 34,970–972.
[21] Arya S., Mishra D.K. and Bornare S.S. (2013) *The Bioscan*, 8(4), 1193-1196.
[22] Bates L.S., Waldren R.P. and Teare I.D. (1973) *Plant and Soil,* 39,205–207.
[23] Panse V.G and Sukhatme P.V. (1978) *Statistical methods for agricultural workers ICAR, New Delhi,* 145-150.
[24] Pearson K. (1895) *Notes on regression and inheritance in the case of two parents,Proceeding of the Royal Society of London,* 58, 240-242.
[25] Delauney A.J. and Verma D.P.S. (1993) *The Plant Journal,* 4, 215–223.
[26] Ramanjulu S. and Sudhakar C. (2000) *Journal of Plant Physiology,* 157,81-85.
[27] Nayyar H. (2003) *Environ Exp Bot.,* 50,253–64.
[28] Zhu X., Gong H., Chen G., Wang S. and Zhang C. (2005) *J Arid Environ*., 62, 1–14.
[29] Chen W. and Li P. H. (2002) *Plant Cell Environ.,* 25, 955–62.
[30] Parvanova D., Ivanov S., Konstantinova T., Karanov E., Atanassov A. and Tsvetkov T. (2004) *Plant PhysiolBiochem.,* 42,57–63.
[31] Aziz A., Martin-Tanguy J. and Larher F. (1999) *Plant Science,* 145, 83–91.
[32] Munns R. and Tester M. (2008) *Annual Review Plant Biology,*59, 651-681.
[33] Chen Z., Cuin T.A., Zhou M., Twomey A., Naidu B.P. and Shabala S. (2007) *Journal of Experimental Botany,* 58, 4245-4255.
[34] Hoque M.A., Banu M.N., Nakamura Y., Shimoishi Y. and Murata Y. (2008) *Journal of Plant Physiology*, 165, 813-824.
[35] Ayerbe L. and Tenorio J.L. (1998) *Field crop Res*., 59, 225-235.
[36] Serraj R. and Sinclair T.R. (2002)*Plant cell Environ.,* 25, 333-341.
[37] Smith B.N., Girija C. and Swamy P.M. (2002) *Environ.and Exp. Bot.,* 47, 1-10.
[38] Huang Z., Zhao L., Chen D., Liang M., Liu Z., Shao H. and Xiaohua Long X. (2013) *PLoSONE,* 8(4), e62085.
[39] Kumar S.G., Reddy A.M. and Sudhakar C. (2003) *Plant Science,* 165,1245-1251.
[40] Hui Z., Tian F.X., Wang G.K., Wang G.P. and Wang W. (2012) *Plant Cell Rep,* 31, 1073-1084.
[41] Yin X.G., Luo Q.X. and Wang W.Q. (2001) *Southwest China Journal of Agricultural Science*, 14, 62-65.