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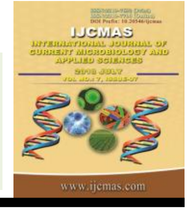

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Role of Glycine Betaine and Potassium Nitrate in Drought Tolerance using Proline Accumulation in Rice (*Oryza sativa* L.)

Nitish Sharma[1], A.K. Singh[1], Nikita Nehal[1*], Krishna Kumar Mishra[2] and Mayanker Singh[1]

[1]Department of Crop Physiology, [2]Department of Post-Harvest Technology, NDUA&T, Kumarganj, Faizabad-224229, India

**Corresponding author*

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A B S T R A C T

Present investigation was carried out in the rainout shelter at Student Instructional Farm (SIF), Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.) during *Kharif* (wet season) 2015 and 2016. Experiments were laid out in randomized block design with 3 replications, one variety *i.e.* Swarna *Sub 1*and nine foliar treatments. Rice plants were exposed to drought at 60 DAT for 15 days by holding irrigation during drought treatment. During drought treatment soil moisture tension of the field was ranged from 60-80 kPa. Foliar application of different concentrations of glycine betaine (100 and 200 ppm) applied at 60 DAT and different concentrations of KNO_3 (2 and 3%) applied at 30 DAT and at 60 DAT as well as their combination increased chlorophyll, proline content in leaves. Increased proline accumulation with foliar treatments during drought provides resistance against reactive oxygen species and protected the quaternary structure of proteins thus prevented oxidative damage to membranes and enhanced Antioxidative defense system under osmotic stress and ultimately produces higher test weight as compared to control with distilled water treatment and exposed to drought. However, among all the treatments, T_7 (foliar spray of glycine betaine @ 200 ppm at 60 DAT) showed maximum chlorophyll content while maximum proline content and test weight found in T_9(foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 200 ppm at 60 DAT) during both the years. Thus, it may be concluded that combination of KNO_3 @ 2% applied at 30 DAT and glycine betaine @ 200 ppm applied at 60 DAT can be used to improve test weight under drought at flowering stage in rice by enhancing proline accumulation.

Introduction

Rice (*Oryza sativa L.*) is a major staple food crop in many parts of the world, feeding more than three billion people and providing 50-80 % of their daily calories intake (Khush, 2005). It is a drought susceptible crop exhibiting serious deleterious effects when exposed to water stress at critical growth stages especially at reproductive stage (Suriyan *et al.*, 2010). Drought is one of the major abiotic stresses that's everely affect and reduce the yield and productivity offood crops worldwide up to 70% (Kaur *et al.*, 2008; Thakur *et al.*, 2010;

Akram *et al.,* 2013). The response of plants to drought stress is complex and involves changes in their morphology, physiology and metabolism. Reduction of plant growth is the most typical symptom of drought stress (Sairam and Srivastava, 2001).

Increased production of compatible solutes (known as osmolytes) in response to drought stress is generally observed in a variety of plants. These compatible solutes are highly soluble organic compounds, with nontoxic nature at higher concentration and carry no net charge at physiological pH. Further, these are accumulated in form of betaines, polyols, oligosaccharides, aminoacids etc. in higher plants. Glycinebetaine (GB) is one such osmolyte whose association with tolerance to abiotic stresses has been supported by a number of publications (Manaf, 2016; Wang *et al.*, 2010; Park *et al.*,2007).The researches on manipulation of GB biosynthetic pathway by genetic transformation and exogenous application are in continuous progress. However, exogenousfoliar application of GB represents a short and simple approach for mitigating the adverse effects of stress.

According to IPIOUAT-IPNI Intern Symposium (2009) mineral-nutrient status of plants has major role in its adaptation to stress. K plays a vital role in improving the plant resistance. Kregularizes physiological processes like photosynthesis, translocation of cations into sink organs, regulation of turgor pressure and enzymes activation (Mengel andKirkby, 2001). During stress condition, ROS formation was induced andoxidative damage to cells occurred and requirement for K was increased (Foyer *et al.,* 2002). This enhanced need for K by plants suffering from drought stress showed that K is required for photosynthetic and CO_2 fixation, because water deficit caused stomatal closure and decreased the CO_2 fixation. Mengel and Kirkby (2001) observed that due to low K concentration, ROS production was induced during water deficit which caused disturbance in stomatal opening. Low grain yield resulting from water deficit could be overcome by increasing K supply (Damon and Rengel, 2007). Results reviewed in this section indicate that under water limited conditions, yield losses can be minimized by the sufficient supply of K.

Materials and Methods

The present investigation was carried out in the rainout shelter (25 m length and 7.5 m width) of the Student Instructional Farm (SIF), Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.) during *Kharif* (wet season) 2015 and 2016. Experiments were laid out in randomized block design with three replication and one variety *i.e.* Swarna *Sub 1*. Twenty five days old seedlings were transplanted in the rainout shelter. At 60 DAT plants were exposed to drought by holding irrigation for 15 days and rainout shelter was properly covered with the polythene to avoid the rainwater during the drought treatment. During 15 days of drought treatment soil moisture tension was measured and it was ranged from 60-80 kPa, after 15 days of drought treatment field was reirrigated to release drought. The treatments comprised of T_1 (Control- Distilled water spray), T_2 (foliar spray of KNO_3 @ 2% at 30 DAT), T_3 (foliar spray of KNO_3 @ 3% at 30 DAT), T_4 (foliar spray of KNO_3 @ 2% at 60 DAT), T_5 (foliar spray of KNO_3 @ 3% at 60 DAT), T_6 (foliar spray of glycine betaine @ 100 ppm at 60 DAT), T_7 (foliar spray of glycine betaine @ 200 ppm at 60 DAT), T_8 (foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 100 ppm at 60 DAT) and T_9 (foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 200 ppm at 60 DAT).Chlorophyll and proline were recorded at before and after drought and test weight was recorded after harvesting. Chlorophyll content of leaf was directly measured in intact leaves with the help of microprocessor based plant efficiency analyzer model: X55/M-PEA. Third leaf from the top was taken for this purpose. Free proline content in leaves was estimated spectro-photometrically according to the methods of Bates *et al.,* (1973).1000-grains were counted from the samples of each treatment. These counted grains were weighed and recorded as test weight at 15% moisture level.

Results and Discussion

Data pertaining to chlorophyll content in leaf, influenced by foliar spray of different concentrations of glycine betaine and KNO_3 (Osmoprotectants) alone as well as their combination applied at different stages on rice plants exposed to drought stress at flowering stage (60 DAT) recorded at different growth stages have been presented in Table 1. At 60 days after transplanting (before drought treatment), among all the treatments significant increase in chlorophyll content was recorded in T_3 followed by T_2, T_8 and T_9 in year 2015-16 while rest of the treatments *viz*., T_4, T_5, T_6 and T_7 showed non-significant effect over T_1. Likewise in year 2016-17, T_3 registered significant increase in chlorophyll content followed by T_9, T_2 and T_8 while rest of the treatments *viz*., T_4, T_5, T_6 and T_7 showed non-significant effect over T_1. At termination of drought (*i.e.* at 75 days after transplanting) show significant increase in chlorophyll content during both the years. However in year 2015-16 among the treatments, maximum chlorophyll content was recorded in T_7 followed by T_8, T_6, T_4 and T_2 while minimum was noted in T_1. Similarly in year 2016-17, maximum chlorophyll content was recorded in T_7 followed by T_8, T_9, T_5, $T_{3,}$ T_6, T_4 and T_2 while minimum was noted in T_1.Chlorophyll is one of the major components to determine the yield as it is a photosynthetic pigment and helps in the net photosynthesis process. Under drought stress reduction in chlorophyll content is common. In the present study, the effect of drought stress on chlorophyll content is mitigated with the foliar application of glycine betaine and potassium nitrate. The result indicates that foliar spray of glycine betaine and KNO_3 maintained higher chlorophyll content under drought, might be because of the role of solute in protecting the photosynthetic machinery from oxidative damage Cha-um *et al.,* (2013). Similar results were also found by Shallan *et al.,* (2012)who reported that exogenous application of solutes like glycine betaine, putrescine etc. ameliorate the negative effect of drought by preventing photosynthetic machinery.

Data regarding proline content, influenced by the foliar spray of different concentrations of glycine betaine and KNO_3 (Osmoprotectants) alone as well as their combination applied at different stages on rice plants exposed to drought stress at flowering stage (60 DAT) recorded at different growth stages have been presented in Table 2. Data obtained at 60 days after transplanting (before drought treatment) showed that among all the treatments, maximum proline content was recorded in T_3 followed by T_9, T_8, T_2 while rest of the treatments *viz*., T_4, T_5, T_6, T_7showed non-significant effect over T_1in year 2015-16 and 2016-17 respectively. Moreover, proline content recorded at termination of drought (*i.e.* at 75 days after transplanting) showed that all the treatments significantly increased the proline content during both the years (2015-16 and 2016-17). However in both the year among the treatments, maximum proline content was recorded in T_9 followed by T_8,T_7, T_5, T_3, T_6, T_4, $T_2$and while minimum was noted in T_1. Similarly in year 2016-17 among the treatments, maximum proline content was recorded in T_9 followed by T_8, T_7, T_5, T_3, T_6, T_2 and T_4 while minimum was noted in T_1.Proline has been assigned the role of cyst solute, a storage compound or a protective agent for cytoplasmic enzymes and cellular structure (Pandey and Ganapathy, 1985). Hanson and Hits (1982) suggested that proline accumulation is a consequence of stress induced damage to cells. In plants, the role of proline may not be restricted to that of compatible osmolytes, but proline synthesized during water deficit and salt stress may serve as an organic nitrogen reserve that can be utilized during recovery (Trotel *et al.*, 1989).These results are in agreement with the findings of Farooq *et al.*, (2008), Anjum *et al.*, (2012) and Zhang *et al.*, (2013) who reported that exogenous application of glycine betaine and potassium increased the proline content which can be utilized during recovery and thereby helps to reduce damage to plant cells and to maintain membrane integrity.

Table.1 Effect of foliar spray of glycine betaine and KNO_3 on chlorophyll content (SPAD Value) in leaves of rice plants exposed to 60-80 kPa drought stress at 60 DAT

Stage → Treatments ↓Year →			60 DAT		75 DAT	
			2015-16	**2016-17**	**2015-16**	**2016-17**
T_1	:	**Untreated**	12.31	13.47	10.22	11.20
T_2	:	**Foliar spray of KNO_3 @ 2% at 30 DAT**	14.44	15.27	11.17	12.09
T_3	:	**Foliar spray of KNO_3 @ 3% at 30 DAT**	15.17	15.91	11.74	12.65
T_4	:	**Foliar spray of KNO_3 @ 2% at 60 DAT**	12.77	13.09	11.30	12.27
T_5	:	**Foliar spray of KNO_3 @ 3% at 60 DAT**	12.34	13.71	12.09	12.71
T_6	:	**Foliar spray of glycine betaine @ 100 ppm at 60 DAT**	11.97	12.99	11.87	12.64
T_7	:	**Foliar spray of glycine betaine @ 200 ppm at 60 DAT**	12.78	13.09	13.65	14.02
T_8	:	**Foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 100 ppm at 60 DAT**	14.11	15.07	12.82	13.27
T_9	:	**Foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 200 ppm at 60 DAT**	13.92	15.57	12.74	12.96
SEm±			**0.26**	**0.31**	**0.26**	**0.28**
CD at 5%			**0.79**	**0.94**	**0.79**	**0.84**

Table.2 Effect of foliar spray of glycine betaine and KNO_3 on proline content(µg g^{-1} fresh weight) in leaves of rice plants exposed to 60-80 kPa drought stress at 60 DAT

Stage → Treatments ↓ Year →			60 DAT		75 DAT	
			2015-16	2016-17	2015-16	2016-17
T_1	:	Untreated	341.69	356.77	449.83	468.50
T_2	:	Foliar spray of KNO_3 @ 2% at 30 DAT	385.67	404.74	490.56	514.10
T_3	:	Foliar spray of KNO_3 @ 3% at 30 DAT	412.05	428.38	544.95	551.44
T_4	:	Foliar spray of KNO_3 @ 2% at 60 DAT	347.98	360.33	491.40	504.71
T_5	:	Foliar spray of KNO_3 @ 3% at 60 DAT	345.46	354.26	547.79	559.07
T_6	:	Foliar spray of glycine betaine @ 100 ppm at 60 DAT	349.23	347.98	523.73	540.64
T_7	:	Foliar spray of glycine betaine @ 200 ppm at 60 DAT	356.77	356.77	551.11	577.80
T_8	:	Foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 100 ppm at 60 DAT	388.18	409.53	561.42	582.21
T_9	:	Foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 200 ppm at 60 DAT	392.05	414.66	592.34	621.70
SEm±			7.30	7.83	11.43	11.90
CD at 5%			21.89	23.46	34.25	35.69

Table.3 Effect of foliar spray of glycine betaine and KNO_3 on test weight (g) of rice plants exposed to 60-80 kPa drought stress at 60 DAT

Treatments ↓ Year →			Test weight (g)	
			2015-16	2016-17
T_1	:	Untreated	15.80	16.37
T_2	:	Foliar spray of KNO_3 @ 2% at 30 DAT	17.11	17.91
T_3	:	Foliar spray of KNO_3 @ 3% at 30 DAT	17.32	18.24
T_4	:	Foliar spray of KNO_3 @ 2% at 60 DAT	17.57	18.39
T_5	:	Foliar spray of KNO_3 @ 3% at 60 DAT	17.89	18.84
T_6	:	Foliar spray of glycine betaine @ 100 ppm at 60 DAT	18.12	19.09
T_7	:	Foliar spray of glycine betaine @ 200 ppm at 60 DAT	18.97	19.47
T_8	:	Foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 100 ppm at 60 DAT	19.54	19.94
T_9	:	Foliar spray of KNO_3 @ 2% at 30 DAT and glycine betaine @ 200 ppm at 60 DAT	19.82	20.22
SEm±			0.40	0.41
CD at 5%			1.19	1.22

It is clear from the data presented in Table 3 that all the foliar spray of different concentrations of glycine betaine and KNO_3 (Osmoprotectants) alone as well as their combination applied at different stages on rice plants exposed to drought stress at flowering stage (60 DAT) significantly increased the test weight under drought stress.Data reveal that all the treatments significantly increased test weight under flowering stage drought during both the years (2015-16 and 2016-17). However among the treatments, maximum test weight *i.e.* 19.82 and 20.22 g was recorded in T_9 followed by T_8, T_7, T_6, T_5, T_4, T_3, T_2 while minimum test weight was found in T_1 in year 2015-16 and 2016-17 respectively. Glycine betaine and potassium nitrate treatment might have improved yield performance of rice under drought stress possibly by better net photosynthetic assimilation (Gupta and Thind, 2015; Kausar *et al.*, 2014).

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