International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(3): 797-801

Received: 13-03-2017
Accepted: 14-04-2017

Shri Om Verma
Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

SK Pandey
Department of Plant Breeding & Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Anubha Upadhyay
Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

SD Upadhyaya
Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

AS Gontia
Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Correspondence
Shri Om Verma
Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Biochemical changes in chickpea seed by priming and foliar application of salicylic acid and water under drought and excessive moisture stress

Shri Om Verma, SK Pandey, Anubha Upadhyay, SD Upadhyaya and AS Gontia

Abstract
The present investigation focused on biochemical changes in JG11 and JG 14 seed by salicylic acid (SA) and Hydro-Priming with foliar application of SA and water in the drought and excessive moisture stress (EMS). Nitrogen and Protein content (%) was significantly high in JG11 (Irrigated) and JG14 (drought). Although nitrogen, protein, carbohydrate percent level was changed in JG11 and JG14 beneath drought and excessive moisture stress (EMS). However, JG11 showed high proline level (1.10 $\mu molg^{-1}$). Although JG14 expressed significant response on seed yield $plant^{-1}$ under drought (8.71%) by the priming of salicylic acid (SA) with a foliar spray of 500nm SA in drought.

Keywords: Chickpea seed, Nitrogen, Protein, Fiber, Carbohydrate, Proline, Drought, EMS.

Introduction

Chickpea (*Cicer arietinum* L.) is a cool season legume crop and is grown in several countries worldwide as a food source. In India during 2013-14 chickpea was cultivated in an area about 9.93 million hectares with a production about 9.53 million tones and 960 $kgha^{-1}$ productivity. In Madhya Pradesh during 2013-14chickpea was cultivated in an area of about 3.16 million hectares with production 3.29 million tones and 1044kg/ha. Productivity Chickpea seeds contain on an average 23% protein, 64% total carbohydrates (47% starch, 6% soluble sugar), 5% fat, 6% crude fiber and 3% ash. High mineral content has been reported for phosphorus (340 mg100 g^{-1}), calcium (190mg100g^{-1}), magnesium (140mg100g^{-1}), iron (7mg100g^{-1}) and zinc (3 mg100g^{-1}). (Anonymous 2015) [2]. According to Healthy Eating Index (HEI-2005) scores Consumers of chickpeas and/or hummus have been shown to have higher nutrient intakes of dietary fiber, polyunsaturated fatty acids, vitamin A, vitamin E, vitamin C, folate, magnesium, potassium, and iron as compared to non-consumers by Walace *et al*. (2016) [23].
Chickpea also improves the soil fertility due to its nitrogen-fixing ability (Maiti 2001) [13]. Mandavia *et al*. (2010) [14] reported that lower concentration of salicylic acid (50 ppm) was significantly increased protein content as compared to control at both vegetative and reproductive stages. Crop under stress condition changes in biochemical level along with a reduction in grain yield (Anonymous, 2013) [1]. In chickpea genotype protein percentage was recorded maximum in genotype ICC 4958 and Tyson. Whereas, the minimum was recorded in JG 315 and DCP 92-3 on the treatment of SA@1.5 mM by Patel and Hemantaranjan (2013) [18]. Water deficit plant showed reduces the growth due to influencing cell turgor, stomatal closure and enzymatic changes which are directly control by water potential by El-tayeb, (2005) [8]. Salicylic acid, as an endogenous phenolic growth regulator, adjusts the activity of antioxidant enzymes, enhances plant resistance to abiotic stresses (drought and EMS) Hayat *et al* (2010) [10]. Plant phenolic compounds are the most abundant and important group of defensive compounds that mediate plant defense that polyphenol level was significantly higher in plants than seeds and the highest rate of this compounds in the plant was observed with 0.5 mM SA reported by Boukraâ *et al* (2015) [5] The experiment focused on biochemical changes in percent viz. nitrogen, protein carbohydrate and fiber along with osmolyte (Proline) level in JG11 and JG14 chickpea genotype priming with salicylic acid (SA) and hydro-priming, foliar application SA of and water under drought and EMS.

Materials and Methods

The experiment was conducted Department of Plant Breeding and Genetics, College of Agriculture Jawaharlal Agriculture University Jabalpur (Madhya Pradesh) during Rabi Season year of 2015-16.In Madhya Pradesh, Jabalpur is situated at 23^0 90' N latitude and 79^0 58' E longitudes at an altitude of 411.78 m above the mean sea level. The Experimental field soil was classified as "vertisol" per US classification of soil. The soil of the region has medium to a deep depth and black with sandy clay- loam texture and neutral soil reaction. It swells by wetting and shrinks by drying. Therefore, deep and wide cracks develop during the winter season. The experimental field having a gentle slope, proper drainage and uniform were selected. The soil reaction was neutral to mildly alkaline with an average pH of 7.51. The soil was medium in available nitrogen (252 kg/ha), medium to high in available phosphorus (164 kg/ha) and medium in available potassium (351kg/ha). The soil indicated 47 cm field capacity and 20.5 cm of water point on the volumetric basis. At present experiment was formulated factorial randomized block design (FRBD), two genotype JG11 (V_1) and JG14 (V_2) and four treatments combination (04) (seed priming and foliar application).For seed priming during inhibition phase of germination, biochemical processes initiate. A number of repair mechanisms needed for seed germination and activated, In present investigation adopted seed priming with SA and H_20 (water).Salicylic acid (SA) Priming: (i) Chickpea seed shocked @500nM SA over night at 15^0C. (ii)Hydro - priming: Chickpea seed shocked at the normal water (H_2O) at room temperature overnight.

The combination of treatments were illustrated by Normal recommended application (T0), Drought + Salicylic acid priming + Foliar spray of 500nm salicylic acid at 15 days interval from35 DAS till podding (T1), Excessive moisture (Irrigation started 35DAS) + Salicylic acid primed + Foliar spray of 500nm salicylic acid at 15 days interval from 35DAS till podding (T2), Drought + Hydropriming + Foliar spray of water at 15days interval from 35DAS till podding (T3) and Excessive moisture + Hydropriming + Foliar spray of water at 15 days interval from 35DAS till podding (T4) with three replication (03). Planted seed Row to row spacing and plot size area were maintained by 30cm and 3.6 m ^{2}respectively.Various biochemical quantify from seed *viz.*nitrogen, protein, carbohydrate and fiber and proline content.

Biochemical quantification

Chickpea seed was analyzed for the biochemical constituents at different treatment combination as follows:

a) Protein and nitrogen (%)

The protein contents of was quantified from the mature seeds of chickpea JG11 and JG 14 as per Micro - Kjell dhal procedure (AOAC, 1965)

Nitrogen and protein content were calculated as per following formula:

$$\text{Nitrogen (\%)} = \frac{\text{Normality of } H_2SO_4 \text{ x Volume of } H_2SO_4 \text{ x } 1.4}{\text{Weight of sample}} \text{ X100}$$

Protein (%) = Percent of nitrogen x 6.25

b) Total carbohydrates (%)

Total carbohydrates percent level was estimated from mature seeds of chickpea JG11 and JG 14by the method of hydrolysis as in AOAC (1984).Calculate the amount of total carbohydrate present in the chickpea seed (sample)solution using the standard graph.

$$\text{Total carbohydrate in the sample} = \frac{\text{Sugar value from the graph (μg)}}{\text{Aliquot sample used}} \text{ x } \frac{\text{Total volume of extract (100 ml)}}{\text{Weight of sample (100mg)}} \times 100$$

c) Proline (µmol/g)

Estimation of proline from chickpea seed (JG11 and JG14) were determined by the method of (Bates *et al*., 1972) [3]. The protocol was based on the formation of red coloured form azone by proline with ninhydrin in acidic medium, and concentrate of proline was estimated by referring to a standard curve made from known as the concentration of proline.

Statistical analysis

Statistical analysis was performed with Windostat version 9.1 analytical software at the 5% probability level.

Results

Chickpea seed expressed protein (%) was recorded maximum in JG11 T_2 (25.42 %) and carbohydrate showed the maximum in JG11T_4 (62.52 %). However, seed yield plant^{-1} was observed maximum in JG 11 T_4 (41.41gplant^{-1}) combination. The protein (%) was significantly superior in JG11 T2, however, JG11 T0, T_1, T_3 and JG14 T_2, T_3 combination showed statistically at par and lowest protein was recorded in JG14 T4 (17.52%). although JG14 T0 T1 T4 and JG11 T4 expressed statistically at par. In case carbohydrate showed the superior significant result in JG11 T_4 combination but JG 11 T_2 T_3 and JG14 T_0, T_3, T_4 expressed statistically at par, however, JG11 T_0 T_1 and JG14 T_1 T_2 combination showed statistically at par and lowest showed in JG11 T1(59.26%). Seed yield (g) plant-^{1} was recorded significantly superior in JG11 T_4 combination. However, JG14 T_0 and JG11 T_1 expressed statistically at par JG14 T_1 T_2 T_3 and JG11 T_3 showed statistically non-significant. However JG14 T_2 JG11 T_0 T_2 and JG14 T_4 expressed statistically at par and minimum seed yield plant^{-1} showed in JG11T_0 (21.01 g Plant-^{1}). (Table - 1). Nitrogen (%) was significantly superior in JG11 T_2 and JG11 T_3 (Figure 1). However JG14 superior nitrogen (%) showed in T_3 than T_2 combination. Graphical data expressed (a), (ab) and (abc) combination showed statistically at par. Although the nitrogen (%) was observed minimum in JG14 T_4 combination (e) (Figure 1). Proline was significantly superior in JG11 T_3 (1.10 µmolg^{-1}) among the treatment. JG14 showed the higher concentration level in JG14 T_2 (1.07 µmolg^{-1}) combination. The minimum proline level was expressed in JG11 T_0 (0.96 µmolg^{-1}).

Table 1: Effect of salicylic acid (SA) and water treatment on chickpea seed the level of protein, carbohydrate and seed yield plant^{-1} under drought and excessive moisture stress.

	Protein (%)	Carbohydrate (%)	Seed Yield (g)Plant^{-1}
JG11 To	22.77	60.05	21.01
T1	23.73	59.26	29.11
T2	25.42	60.56	23.60
T3	24.38	61.64	25.45
T4	19.83	62.52	41.41
JG14T0	19.36	62.44	32.62
T1	20.81	60.37	27.09
T2	22.67	59.73	24.73
T3	23.42	60.84	28.58
T4	17.52	60.76	21.90
Mean	21.99	60.82	27.55
C.V.	8.69	1.96	7.52
S.E.	1.10	0.69	1.20
C.D. (5%)	3.28	2.05	3.55
Range Lowest	17.52	59.26	21.01
Range Highest	25.42	62.52	41.41

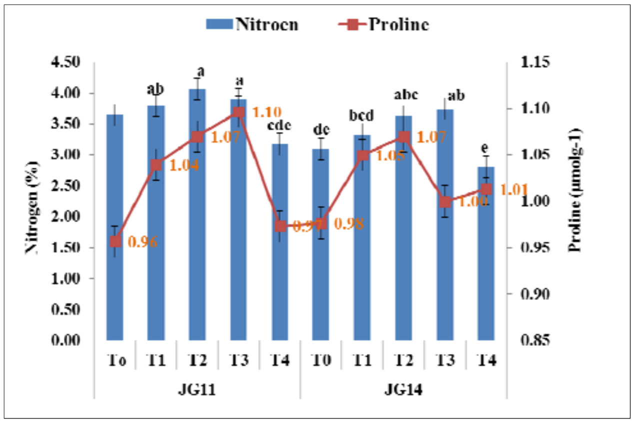

Fig 1: Effect of salicylic acid (SA) and water treatment on chickpea seed nitrogen content and proline level under drought and excessive moisture stress.

Discussion

The plant performance is attributed to the genetic factors which are controlled by the differences in the biochemical parameters. Plant growth regulator (PGR) and water spraying have been shown to influence these parameters in one way or the other. The seed nitrogen content (%), protein (%), carbohydrate (%) and proline (µmolg^{-1}) in chickpea were significantly varied due to foliar application of salicylic acid and water.

Nitrogen (%)

Mineral nutrition is a basic requirement for proper growth and development and survival under different environmental stress conditions. Studies have shown that mineral nutrient status in chickpea plays a critical role in the alleviation of abiotic stress similar repot expressed by (Syeed *et al*. 2011) [21]. SA-mediated changes in photosynthesis were attributed nutrients Nitrogen content in *B. juncea* cultivars differing in salt tolerance (Nazar *et al*. 2015) [15]. The present study indicated (Figure 1) that genotype JG11 possessed maximum nitrogen (4.06 %) in Salicylic acid primed seed + Foliar spray of 500nm salicylic acid at 15 days interval combination. However, JG14 registered the lowest value (2.80 %). Although excessive moisture stress + Hydropriming of seed + Foliar spray of water at 15 days interval showed the lowest nitrogen content. Brijnandan *et al*. (2014) [6] reported that seed priming and foliar application of 2% urea at flower initiation stage the highest nitrogen content (3.09%) in chickpea.

Protein (%)

These findings are in conformity with the results of Mandavia *et al*. (2010) [14] lower concentration of salicylic acid (SA 50 ppm) was significantly increased protein content as compared to control at both vegetative and reproductive stages. The protein percent was increased in genotype JG11 (25.42%) in Salicylic acid primed seed + Foliar spray of 500nm salicylic acid at 15 days interval combination and significantly dominated over JG14 (Table 1). These findings confirm to Venkates and Basu (2011) [22]. Similar finding expressed under the normal and stress condition the maximum protein percentage was observed in the genotype ICC 4958 (28.3 and 21.3%) followed by genotype Tyson (25.3 and 18.6%) whereas, the minimum was noticed in JG 315 (23.3 and 15.6%) and DCP 92-3 (21.9%, 14.3%) in the treatment of SA@1.5 Mm in chickpea (Patel and Hemantaranjan 2013) [18].

Carbohydrate (%)

Application of salicylic acid, glutathione and paclobutrazol were significantly increased total carbohydrates of the produced seed reported by Bekheta and Talaat (2009) [4]. The present investigations carbohydrate percent in genotype JG11 (62.51 %) significantly dominated over JG14. The highest carbohydrate (%) expressed in excessive moisture and seed hydropriming with Foliar spray of water at 15 days interval (Table 1). By infusion of sucrose into the stem, demonstrated that low assimilate supply contributes to reproductive in chickpea reported by Khan *et al*. (2016) [12, 16]. Similar finding expressed by Singh *et al* (2016) [20] that Sugar content was significantly increased with salicylic acid treatment @1.0mM.

Proline (µmolg^{-1})

The chickpea seed proline content showed the maximum in JG 11 (1.09 µmolg^{-1}) in Drought + Hydropriming + Foliar spray of water at 15 days interval (Figure 1).The present level of proline in seed also responsible for the surviving the seed, with the presence of SA stimulate the level of proline under abiotic stresses. Similar finding explained the plants treated with salicylic acid at the rate of 1.5 mM under drought stress maximum [310.67 µg g^{-1}] in Tyson and minimum in DCP 92-3 [188.0 µg g^{-1}] in chickpea (Patel and Hemantranjan 2012) [17]. The application of SA increased the activity of proline content, where the maximum response was generated in the plants sprayed with 10^{-5} mol/L of SA, showing a significant increase of 43.1% (proline) over control in chickpea (Hayat *et al*. 2012) [11]. However, the salicylic acid spraying in complete drought stress condition significantly increased the proline content in chickpea (Farjam *et al*. 2014) [9].

Seed yield (g plant^{-1})

Seed yield in legumes depends on a number of seeds plant^{-1}. It has been observed that if an attempt is made to increase one component, there is compensatory decreased in other component and yield remains more or less same suggesting that there is some mechanism operating in plant system which is acting as constraints in controlling the productivity. Seed yield plant^{-1} was showed significantly higher in JG11 (41.41gplant^{-1}) at Excessive moisture Stress (EMS) + Hydropriming + Foliar spray of water at 15 days interval (T4) combination. However, in T4 condition SA showed negative responses in presence of moisture available. Higher accumulation of biomass in JG11 and having enlarged internodal length and genotype potentiality through surviving and better responses in EMS. Although reproductive phase has more days and branches prior to bear flower till physiological maturity. However, JG 14 genotypes showed sensitive response under EMS seed yield plant-1 (21.89g). However, seed yield plant-1 in drought (27.09g) and EMS (24.73g) with foliar application of SA showed a significant response in JG14. (Table -1). Similar finding proposed the highest seed yield was recorded by Rajas, Vishal, RCLG-213, RCLG-204 and RCLG-248 in chickpea (Sarde *et al*. 2011) [19]. The GA_3, IAA and salicylic acid increased the seed yield up to 35 % over control (Dawood *et al*. 2012) [7]. Foliar application of 1.2 mM salicylic acid increased grain yield by 25% as compared with control in chickpea. Under EMS and foliar application of water were significantly increased grain yield (due to stage-specific condition exposed irrigation) and having a genotypic potential of chickpea. However, drought situation grain yield was reduced. The similar result reported by Pang *et al*. (2016) [16].

Conclusions

In present investigation mitigate the effect of drought and excessive moisture stress used of seed priming with SA and Water. Showed beneficial effect on abiotic stresses. Osmolite (proline) responsible for plant used as an osmoprotectant and regulate the osmoregulation along with maintaining the homeostasis inside the plant cytoplasm. Seed priming with SA and hydro-priming and foliar application of SA and Water enhanced the activity of proline and nutrient uptake efficiency viz. Nitrogen and Protein content. JG14 seed yield plant^{-1} was increased about drought (8.71%) in compared to the same genotype under EMS. Therefore SA is useful as drought as well as genotypic potential (JG14) beneficial for yield improvement.

Acknowledgements

Authors are grateful thanks to Department of Plant Breeding &Genetics JNKVV Jabalpur and ICAR -AICRP on Chickpea Physiology and IIPR Kanpur is duly acknowledged.

References

1. Anonymous. Agricultural statistics, Agricuture Ministry, Tehran, Iran, 2013.
2. Anonymous. Project Coordinators Report AICRP on Chickpea Annual Group Meet 31ST August to 3rd September at Punjab Agricultural University Ludhiana, 2015.
3. Bates LS, Waldren RP, Teave ID. Rapid determination of proline for water stress studies. Plant and Soil. 1972; 36:205-207.
4. Bekheta MA, Talaat IM. Physiological response of mungbean "*Vigna radiata*" plant to some bioregulators. Journal of Applied Botany and Food Quality. 2009; 83:76-84.
5. Boukraâ D, Belabid L, Benabdelli K, Bennabi F. Implication of salicylic acid in chickpea growth to salt resistance Advance. Environment Biology. 2015; 9:270-277,
6. Brijnandan, Jamwal BS, Sharma BC, Kumar A, Gupta V. Seed priming and foliar nutrition studies on growth, yield and quality of chickpea under subtropical kandi areas of hills of shivalik foothills of Jammu region. International Journal of Basic and Applied Agricultural Research. 2014; 12:184-187.
7. Dawood MG, Sadak MS, Hozayen M. Physiological role of salicylic acid in improving performance, yield and some biochemical aspects of sunflower plant grown under newly reclaimed sandy soil. American Journal Basic Science. 2012; 6:82-89.
8. El-Tayeb MA. Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regulator. 2005; 45:215-224
9. Farjam S, Siosemardeh A, Kazemi Arbat H, Yarnia M, Rokhzadi A. Response of chickpea (*Cicer arietinum* L.) to exogenous salicylic acid and ascorbic acid under vegetative and reproductive drought stress conditions. Journal of Applied Botany and Food Quality. 2014; 87:80-86.
10. Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: A review. Environmental and Experimental Botany. 2010; 68:14-25.
11. Hayat Q, Hayat S, Alyemeni MN, Ahmad A. Salicylic acid mediated changes in growth, photosynthesis, nitrogen metabolism and antioxidant defense system in (*Cicer arietinum* L.) Plant Soil Environmental 2012; 58:417-423.
12. Khan HA, Siddique KHM, Colmer TD. Vegetative and reproductive growth of salt stressed chickpea are carbon-limited: sucrose infusion at the reproductive stage improves salt tolerance. Journal of Experimental Botany. 2016; 1-11
13. Maiti RK. The Chickpea Crop. In Advances in Chickpea Science, Science Publishers Inc., Enfield, USA. 2001; 1-31.
14. Mandavia C, Raval L, Mandavia MK, Khasiya V. Influence of salicylic acid and brassinolide on biochemical composition and yield of chickpea. Indian Journal of Agriculture Biochemistry. 2010; 23:32-35.
15. Nazar R, Umar S, Khan NA. Exogenous salicylic acid improve s photosynthesis and growth through increase in ascorbate-glutathione metabolism and S assimilation in mustard under salt stress. *Plant Signal. Behaviour*. 2015; 10.
16. Pang J, Turner NC, Khan T, Du YL, Xiong JL, Colmer TD, *et al*. Response of chickpea (*Cicer arietinum* L.) to terminal drought: leaf stomatal conductance, pod abscisic acid concentration, and seed set. Journal of Experimental Botany. 2016; 1-13
17. Patel PK, Hemantranjan A. Salicylic acid induces alternation in dry matter partitioning, antioxidant defence system and yield in chickpea (*Cicer arientinum* L.) under drought stress. Asian Journal of Crop Science. 2012; 4:86-102.
18. Patel PK, Hemantaranjan A. Differential sensitivity of chickpea genotypes to salicylic acid and drought stress during preanthesis: effects on total chlorophyll, phenolics, seed protein and protein profiling. The Bioscan. 2013; 8:569-574.
19. Sarde BH, Wagh RS, Deshmukh DV. Morphophysiological evaluation and dry matter distribution in chickpea (*Cicer arietinum* L.) germplasm. Annals of Plant Physiology. 2011; 25:182-187.
20. Singh R, Hemantaranjan A, Patel PK. Effective mitigation of salinity stress by salisylic acid infield pea (*Pisum sativum*) through altering carbohydrate

metabolism and antioxidant enzyme activity at pre-anthesis stage. 2016; 225-228.

21. Syeed S, Anjum NA, Nazar R, Iqbal N, Masood A, Khan NA. Salicylic acid-mediated changes in photosynthesis, nutrients content and antioxidant metabolism in two mustard (*Brassica juncea* L.) cultivars differing in salt tolerance. Acta. Physiology Plant. 2011; 33:877-886.
22. Venkates MS, Basu PS. Effect of foliar application of urea on growth, yield and quality of chickpea under rainfed conditions. Journal of Food Legume. 2011; 24:110-11.
23. Wallace TC, Murray R, Zelman KM. The Nutritional Value and Health Benefits of Chickpeas and Hummus. Nutrients. 2016; 8:766.