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Biochemical characterization of Indian mustard (*Brassica juncea* L.) genotypes in response to moisture stress and irrigation modules

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Abstract

In the current investigation, the impact of moisture stress and irrigation modules was studied on biochemical characteristics in twelve *Brassica juncea* genotypes. Experiment was laid in split plot design with three moisture treatments viz. only pre sowing irrigation (I_0), one irrigation at 35 DAS (I_1) and two irrigations, one at 35 DAS and second at 65 DAS (I_2).Water stress up-regulated the sugars and proline content in the genotypes while it reduced the protein content. Highest amount of total sugars was recorded in K-9-108 (70.3 mg g^{-1} DW), reducing sugars in RLC1 (12.61 mg g^{-1} DW), protein content in MLM-19 and NLM-3 (8.8 mg g^{-1}DW) while highest proline was in NPJ-79 (0.87mg g^{-1} DW) under moisture stress. Genotypes differed significantly for the biochemical constituents and total soluble proteins increased progressively with one and two irrigations.

Key words: *Irrigation modules, moisture stress, proline, reducing sugars, sugars*

Introduction

Water stress is one of the important and limiting factor that affects plant growth and productivity in the arid and semi-arid regions of the world. Drought causes a severe impairment in plant photosynthesis, growth and development and hence limits plant production and performance of crop plants worldwide (Azadeh *et al.,* 2014). Water deficit leads to reduced nutrient uptake by roots and transportation from roots to shoots, due to restricted transpiration and impaired active transport and membrane permeability (Yuncai and Schmidthalter, 2005). Also, the response of plants to water stress differ significantly depending upon intensity and duration of the stress, plant species and plant growth stage (Jaleel *et al*., 2008). In response to water deficit, plants evolve biochemical adaptations and exhibit several alterations in metabolic processes viz. accumulation of low molecular weight sugars, amino acids or betaines which maintain cellular turgor as a consequence of decreased water potential. Therefore, at the cellular level, plants attempt to alleviate the damaging effects of stress by altering their metabolism to cope with stress (Bayoumi *et al.*, 2008).The decreased water availability negatively affects the metabolite concentration, followed by alteration in carbohydrate metabolism and increased synthesis of compatible solutes such as reducing sugars. The organic and inorganic solutes thus accumulated raises the osmotic pressure in the cytosol, thereby maintaining cellular turgor and a driving force for water uptake. The level of sugars generally increases under water for removal of closely associated water from the protein without leading to conformational changes and loss of enzymatic functions (Yordonov *et al.*, 2003). An increase in sucrose and hexose levels under moisture stress has been proposed as the osmotic *sugars*, adjustment in sucrose transporting species (Westgate and Boyer, 1985). On the other hand decrease in protein synthesis causes rapid dehydration. Therefore, changes in protein content is considered as an important response of plants to environmental stress and as an adaptive response towards moisture stress. Earlier reports the positive (Shahraki et *al.*, 2008 , Tohidi *et al.*, 2011) as well as non-significant effect of drought (Praveen *et al*., 1996, Tahir *et al.,* 2007) on protein content which are affected by various factors like variety, class and environmental stress encountered during plant growth and development. Ahmadi et *al*. (2010) reported increased protein content in maize seedlings exposed to mild water stress which decreased on exposure to severe drought. Drought tolerance is an interactive association of complex morphological, physiological and molecular characters associated with low molecular weight biomolecules like proline, the most compatible osmolyte increasing under drought stress and is considered as an important stress tolerance mechanism (Verbruggen and Hermans, 2008). Biochemical alterations and the adaptive response of plants towards water stress tolerance is of great importance for the plant breeders in developing drought tolerant varieties by understanding the detrimental effect of drought on biochemical traits in plants. The present study was therefore undertaken to visualize the alterations in the biochemical characters in Indian mustard genotypes as influenced by moisture deficit and irrigation modules.

Material and Methods

Thirty genotypes of *B. juncea* were evaluated under field conditions of oilseeds farms (30^0 54'N $75^0$48'E 247m) located at Punjab Agricultural University Ludhiana, Punjab, India. Based on their performance twelve genotypes were selected for further study during two consequective years 2009-10 and 2010-11. Experiments were laid down in split plot design in three replications according to recommendations of package of practices. Treatments comprised of irrigation schedule in main plots and genotypes in subplots. Irrigation regimes consisted of only pre-sowing irrigation designated as moisture stress (I_0), one irrigation at 35DAS (I_1, restricted moisture) and two irrigations at 35 and then at 65DAS (I_2, normal moisture).Three plants were tagged per treatment in each replication.3rd and 4th leaf of the main shoot from the tagged plants were collected at 90 DAS. The sampled leaves were oven dried at 60 ^{0}C± 1^0C for 24 hrs and were used for biochemical estimations. Standard protocols were followed to estimate total soluble sugars (Dubois *et al.,* 1956), reducing sugars (Nelson, 1944), total soluble proteins (Lowry et *al.,* 1951) and Proline (Bates *et al.,* 1973). The difference between total soluble sugars and reducing sugars was computed which gave the non-reducing sugar content in the cultivars**.** The CPCS1 software developed at PAU was applied for statistical analysis. The effects were computed at 5% and 1% level of significance.

Results and Discussion

Variations existed between the genotypes studied. The irrigation modules and the interactive effects (genotypes x irrigations) were significant for all the sugar moieties.

***Sugars*:** Total sugar content was highest in cultivar K-9-108 under stress and in cultivar QM-7-196 both under restricted and normal moisture regimes.K-109-113 possessed lowest total sugar content under all moisture regimes (Fig.1).Total soluble sugars acts as osmoprotectant thus maintaining the turgor pressure and stabilizes the cellular membranes. Soluble carbohydrates play a potential role in adaptation to water stress as reported in maize (Mohammadkhani and Heidari, 2008). Increased sugar content under drought stress has been reported in maize by Sinay and Karuwal (2014) and Homayouni and Khazarian (2014) as well in *Brassica napus* (Nosrati *et al*., 2014). RLC1 had highest reducing sugars under all the moisture regimes. Maximum amount of non-reducing sugars were estimated in K-9-108 (62.6 mg g^{-1} DW) under moisture stress while QM-7-196 registered highest content under restricted and normal moisture schedules. K-9-108 had the lowest content of non-reducing sugars under 3 irrigation regimes (Fig.2). Overall, the content of total sugars, reducing and non-reducing sugars was highest cultivars under moisture stress and the decline in respective content was observed with one and two irrigation module.

Total soluble proteins (TSP): Genotypes differed significantly for the total soluble protein content but their content increased with irrigations applied. Cultivars registered maximum soluble proteins with two sequential irrigations given at 35 and 65 DAS.

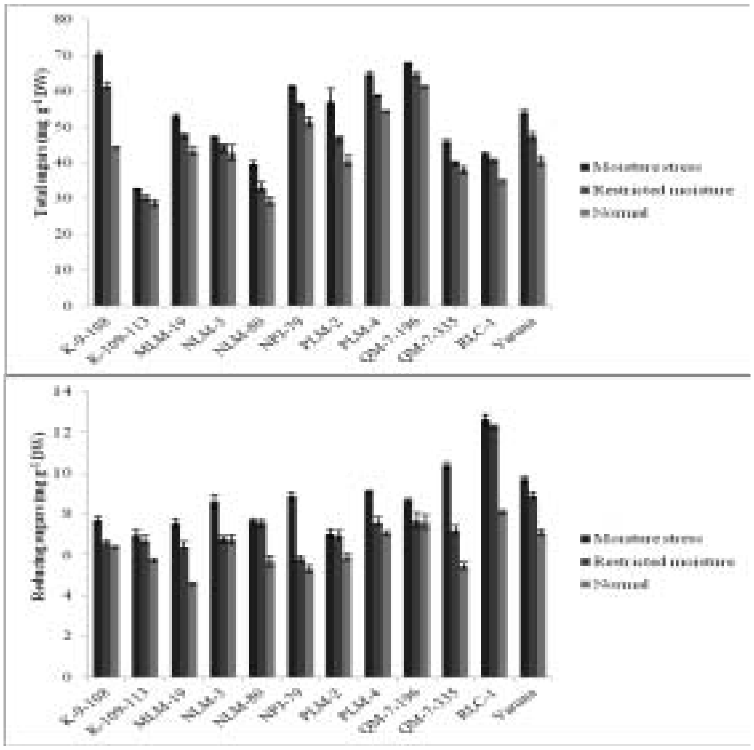

Fig1: Total sugars (TS) and reducing sugars (RS) as influenced by moisture stress and irrigation modules

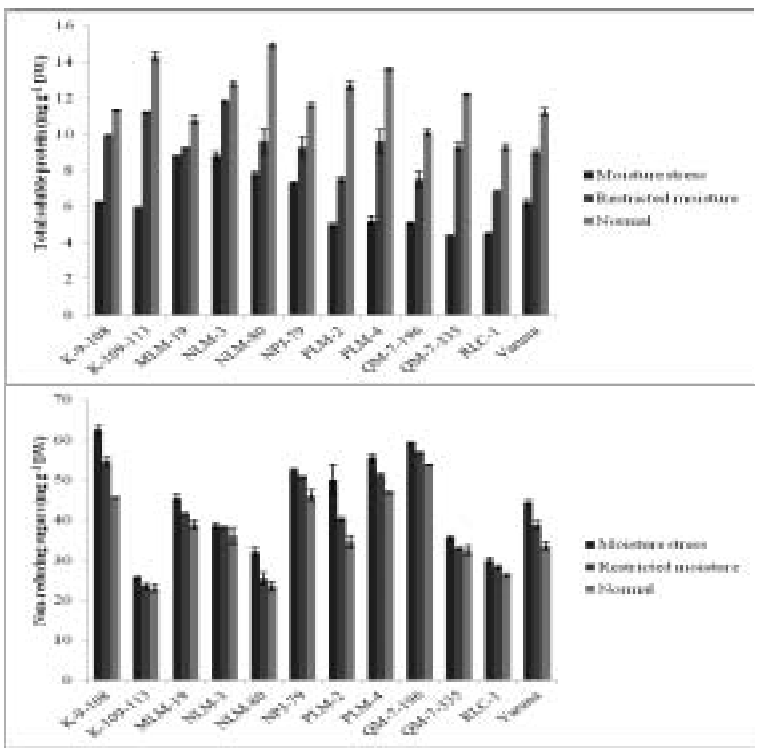

Fig2: Non-reducing sugars (NRS) and total soluble proteins(TSP) under moisture stress and irrigation modules.

Interactive effects between genotype x irrigation were significant too. Cultivars MLM-19 and NLM-3 possessed highest protein content of 8.8 mg g^{-1} DW under moisture stress. NLM-3 registered highest protein content with one irrigation and NLM80 with two irrigations. RLC-1 registered the lowest amount of soluble proteins under all the three moisture regimes (Fig.2). Leaf protein content gradually decreases under moisture deficit conditions (Roy *et al.,* 2009). Further, increased protease enzyme activity under reduced moisture reduces protein content. Active protein breakdown pathway leads to reduced protein content under water stress as reported by Sankar *et al.* (2007) in groundnut. Our results are supported by the findings of Shahraki *et al.* (2008) and Tohidi *et al.* (2011) in *B.napus* where protein content decreased under water stress.

Proline content: Highest content of proline was 0.87 mg g^{-1} DW under moisture stress (I_0) in NPJ-79 with a slight decline of 0.82 mg g^{-1} DW in PLM-4. Under restricted moisture NLM-80 had highest proline content (0.69 mg g^{-1} DW) while PLM-4 accumulated highest proline (0.49 mg g^{-1} DW) under normal moisture regime (Fig.3). Proline, one of the most common and compatible osmolytes in water stressed plants and its metabolism has been studied mainly in response to osmotic stress as reported by Verbruggen and Hermans (2008). Proline has the ability to oppose oxidative stress, an important strategy to overcome adverse effects of moisture stress (Vendruscolo *et al.,* 2007).Further, this molecule acts as a signaling molecule in modulation of mitochondrial functions or can trigger specific gene expression that can be essentially important for recovery of plant from stress as reported by Szabados and Savoure (2009). Proline accumulation therefore is considered as a drought tolerance mechanism which gets activated due to loss of feedback inhibition of proline synthesis which in turns declines the proline oxidation. Proline accumulation hence provides a good screening of drought resistant cultivars under water deficit conditions (Rahdari *et al.,* 2012). Results of the current investigation are corroborated by the findings in *B. napus by* Nosrati *et al.* (2014) and in soybean by Amira and Qados (2014).

Correlation: Correlation studies revealed negative association of seed yield with proline (-0.403) under

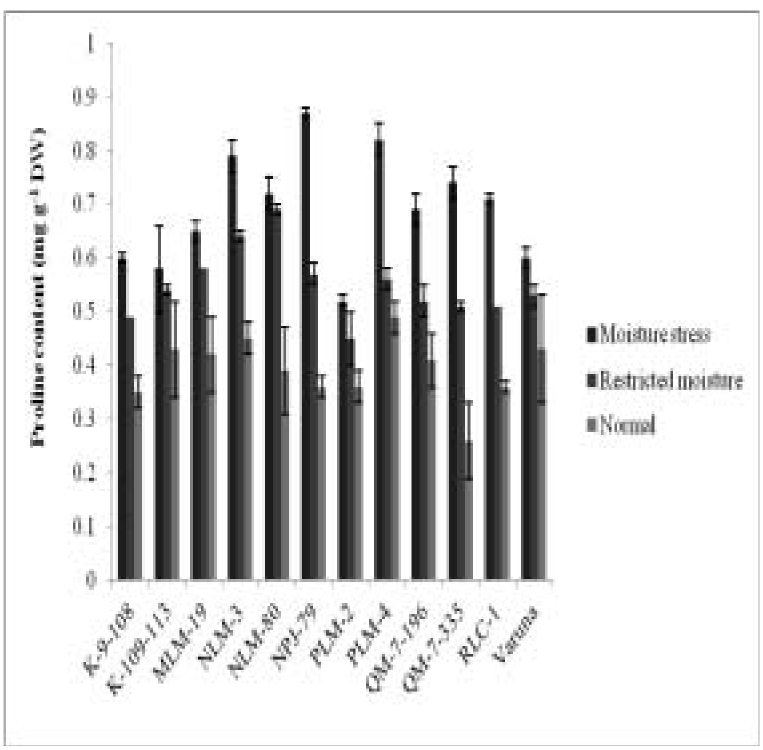

Fig3: Effect of moisture stress and irrigation modules on Proline content of the cultivars.

Table 1: Correlation coefficients of biochemical traits with yield under moisture stress (below diagonal) and restricted moisture (above diagonal)

Parameters	Total Sugars	Reducing Sugars	Non reducing sugars	Total soluble proteins	Proline	DSI_1	DTI_1	DSI_2	DTI_2	Seed yield
	1	2	3	4	5	6	7	8	9	10
Total sugars	1	-0.201	0.989**	-0.256	-0.321	-0.17	-0.171	-0.019	-0.099	0.045
Reducing sugars	-0.103	1	-0.341	-0.568	-0.171	0.422	0.425	0.417	0.115	0.39
Non reducing sugars	0.991**	-0.234	1	-0.162	-0.283	-0.227	-0.228	-0.081	-0.112	-0.015
Total soluble proteins	-0.117	-0.442	-0.055	1	0.527	-0.152	-0.178	0.087	0.002	-0.373
Proline	0.099	0.388	0.046	0.211	1	0.321	0.306	0.217	0.398	-0.253
DSI_1	-0.219	0.253	-0.248	0.276	0.1	1	0.998**	0.652*	0.618*	0.371
DTI_1	0.179	0.017	0.173	-0.172	-0.414	-0.124	1	.645*	0.618*	0.377
DSI_2	-0.059	0.265	-0.093	0.38	-0.114	.652*	0.224	1	-0.046	0.377
DTI_2	0.237	-0.022	0.235	-0.412	-0.203	-0.234	.845**	-0.174	1	-0.315
Seed yield	0.248	-0.099	0.256	-0.258	-0.403	-0.525	.909**	-0.077	.820**	1

DSI 1 and DTI 1: Seed yield at moisture stress (I_0) and restricted moisture (I_1)
DSI 2 and DTI 2: Seed yield at moisture stress (I_0) and normal moisture (I_2).

Table2: Correlation coefficients of biochemical traits with yield under normal moisture regime.

Parameters	Total sugars (TS)	Reducing sugars (RS)	Non-reducing sugars (NRS)	Total soluble proteins (TSP)	Proline	DSI $_1$	DTI $_1$	DSI $_2$	DTI $_2$	Seed yield
	1	2	3	4	5	6	7	8	9	10
Total sugars	1									
Reducing sugars	0.26	1								
Non-reducing sugars		0.968**	0.143	1						
Total soluble proteins		-0.406	-0.398	-0.385	1					
Proline	0.241	0.262	0.157	0.241	1					
DSI $_1$	-0.192	0.046	-0.204	-0.248	0.162	1				
DTI $_1$	-0.192	0.029	-0.206	-0.275	0.148	.998**	1			
DSI $_2$	-0.17	0.304	-0.127	-0.396	0.207	.652*	.645*	1		
DTI $_2$	0.022	-0.081	-0.036	0.248	0.276	.618*	.601*	-0.046	1	
Seed yield	0.145	0.201	0.091	0.143	-0.012	0.364	0.342	-0.179	0.626*	1

DSI 1 and DTI 1: Seed yield at moisture stress (I_0) and restricted moisture (I_1)

DSI 2 and DTI 2: Seed yield at moisture stress (I_0) and normal moisture (I_2).

water stress which narrowed down to (-0.253) under restricted moisture and (-0.012) with normal irrigations (table 1 & 2). Thus, indicating higher amount of osmolytes as an adaptive strategy of plants to cope with water stress. Seed yield was positively and significantly correlated with DTI1 (0.909**) and also DTI_2 (0.820**) under stress and only negatively related with DTI_2 (-0.315) with restricted moisture regime. Positive association existed between seed yield and DTI_2 (0.628*), DTI_1 and DSI_1 (0.988**), DSI_2 and DTI_1 (0.645*) and DTI_2 and DSI_1 (0.618*) with two irrigations.

Conclusion

Overall, water stress lead to increased accumulation of sugars and proline whereas total soluble protein content was reduced in the cultivars. The sugars and proline content in the cultivars was highest under moisture stress, followed by restricted moisture while least was under normal moisture (I_2). The soluble proteins were lowest under water stress and increased substantially with number of irrigation applied. K-9-108, NLM-3 and PLM-2 out yielded other cultivars under moisture stress due to high sugar levels and relatively higher proline content.

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