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PHYSIOLOGICAL RESPONSE OF INDIAN MUSTARD (*BRASSICA JUNCEA* L.) TO DIFFERENT MOISTURE REGIMES

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ABSTRACT
Drought is undoubtedly one of the most important environmental stresses limiting the productivity of crop plants in the arid and semiarid areas of world. The study was carried out during two winter seasons (2009-10 and 2010-11) to investigate the effects of water deficit on leaf water status in terms of RWC and water potential, SPAD chlorophyll, stomatal frequency and different components of chlorophyll fluorescence during different stages of crop growth with drought susceptibility (DSI) and tolerance indices (DTI). Physiological traits were highest at 65DAS and moisture stress reduced SPAD values by 6.1% and 8.6% and RWC by 11.5% and 12.6% at 90 and 120DAS respectively. Profound impact of moisture deficit was to the tune of 52.3% on the mean water splitting capacity on the donor side of PSII (inferred by Fv/Fo) while photochemical efficiency (PSII) was reduced by 4.3% in the *B. juncea* genotypes. Stomatal frequency was higher on the abaxial side. Seed yield (SY) was positively associated with SPAD (0.318) and RWC (0.266) at 90DAS, stomatal size (0.265), Fv/Fo (0.106) and DTI1 (0.429) and DSI3 (0.574*), though the magnitude of association was low under moisture stress. High yielding cultivars under moisture stress *i.e* NPJ-79, NLM-3 and PLM-2 showed comparatively lesser reduction in SPAD, RWC, water potential, disruption of PSII and also water splitting capacity on the donor side of PSII.

INTRODUCTION

Drought is one of the most universal and significant environmental stress affecting plant growth and productivity worldwide. Therefore, understanding crop response to this stress is the basis for regulating crops approximately and achieving agricultural water savings. There are significant differences in the tolerance of plants to drought stress depending upon the intensity and duration of stress, plant species and stage of development (Surendar *et al.,* 2013). The response of a crop to water stress varies with the crop species, crop growth stage, soil type, environment and season. Drought stress causes a series of physiological, biochemical and morphological responses of crops, which finally results in low yield (Sharma *et al.,* 2011; Din *et al.*, 2011). Therefore, insufficient availability of water i.e., drought, is presumably the most common stress experienced by plants responsible for the yield loss in plants (Pedapati *et al.,* 2013; Acharaya *et al.,* 2013). The degree to which plant parts can withstand desiccation is expressed as relative water content (RWC), a better indicator of water stress than other growth parameters. Water deficit is characterized by decrease in RWC and water potential, resulting in wilting, stomatal closure, reduced growth and chlorophyll content. In India, Brassica are mostly grown on light textured soils using water conserved from monsoon rains and inevitably suffer from moisture stress during the reproductive growth when stored water becomes depleted (Ahmadi and Bahrani, 2009). Further, nearly, 85-90% of the total annual rainfall is received during rainy season (June-September). Indian mustard (*B. juncea*) is grown during winter season (rabi) primarily in the marginal lands with limited irrigation or residual soil moisture. In the present scenario, irrigation water is becoming scarce due to its increasing demand for other sectors. There is increasing concern over the effect of climate change on water resources and prudence dictates that water should be used effectively in order to increase and sustain productivity. With the availability of germplasm studies were required to explore the performance of genotypes, assess variation in *Brassica juncea* for drought tolerance and further to identify physiological traits associated for drought tolerance.

MATERIALS AND METHODS

A set of twelve identified genotypes *B. juncea* viz. K-9-108, K-109-113, MLM-19, NLM-3, NLM-80, NPJ-79, PLM-2, PLM-4, QM-7-335, RLC-1 and Varuna were selected for the present investigation, seeds of which were procured from the Oilseeds section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The crop was raised in the experimental area of oilseeds during two rabi (winter) seasons i.e. 30 October 2009 and 4 November 2010. Experiment was laid down in split plot design with three replications according to recommendations of package of practices keeping irrigation in the main plot and genotypes in the sub-plots. For each treatment 4 rows each of 3m row length were sown at 30 cm spacing keeping the plot size of 3.6m^2. 3rd or 4th from top physiologically mature leaf was used for various studies in the present investigation.

Relative leaf water content (RWC)

(Turner, 1986). Discs from five leaves from each treatment were weighed immediately for their fresh weight and then were submerged in 5ml of distilled water in test tubes till saturation. After 4 hrs the discs were removed and the surface water was blotted off with the filter paper without putting any pressure, discs were weighed for saturated weight. After drying the discs at 70 fC for 72 hrs their dry weight was taken. Following formula was used to calculate RWC (%) = Fresh weight-dry weight/saturated weight-dry weight x100

Water potential

Leaf water potential was measured with PSYPRO water potential system (Wescor) in the field. Leaf discs were made with a borer having diameter 6 mm from third or fourth leaf from the main shoot and discs were immediately placed in the disc chamber for 30 seconds to obtain the stable readings.

Stomatal frequency and size

Leaf samples of genotypes were collected at the 120DAS and preserved in Formalin-acetic acid-ethyl alcohol (FAA) solution immediately.

Preparation of FAA solution

Prepared by mixing 85 mL of 50% ethyl alcohol, 5mL of glacial acetic acid and 10mL of 40% formaldehyde.

The preserved leaves were washed thoroughly and excess water was removed by placing the leaf between folds of filter paper. A thin layer of quick fix was applied on both the abaxial (lower) and adaxial (upper) surfaces. The dried film was carefully removed with forceps and mounted on a slide with a drop of water. Cover slip was placed on the film. All sides of cover slip were sealed with nail paint. The slide was focused on the microscope stage (Nikon Eclipse 90i Stereozhoom microscope) and number of stomata was counted by moving the slide in different microscopic areas. All the readings were taken at 20X. The numbers of stomata were counted in ten randomly selected microscopic fields and averaged. Stomatal frequency denotes the number of stomata per microscopic field.

SPAD chlorophyll readings

SPAD meter (SPAD-502) was used for measuring chlorophyll from leaves at 65, 90 and 120DAS.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured with Os30p model by Opti Sciences after the leaves were dark adapted with dark adapting clips. The initial fluorescence (Fo) and maximal fluorescence (Fm) were analyzed and quantum efficiency of open PS II centers-quantum yield (Fv/Fm) calculated. The leaf surfaces were previously adapted to the dark for 15min so that all the centers of PSII were in open stage (all the primary acceptors oxidized) and the energy dissipation through heat was minimal. The Fo was obtained with low intensity light (less than 0.1μmolm^{-2}s^{-1}) not to induce any effect in the fluorescence variable. The Fm was obtained by continuous light excitation (at 2500 μmolm^{-2}s^{-1}) provided by an array of LEDs focused on the leaf surface to provide homogenous irradiation over a 4mm (0.16in) diameter leaf surface. The fluorescence variable (Fv) was calculated from the difference between Fm and Fo.

Drought resistance parameters

Drought susceptibility and tolerance indices were calculated by the formulae of Fischer and Maurer (1987) and Fernandez (1992) respectively. Further DSI1 and DTI was computed between seed yield (SY) at moisture stress and restricted moisture, DSI2 and DTI2 between SY at moisture stress and normal moisture while DSI3 and DTI3 between restricted moisture and normal moisture.

Statistical analysis

Statistical analysis was performed using CPCS1 software in which all the parameters were analyzed for critical difference at 5% level of significance using split plot design program which is also the design of current experiment. Standard errors were also computed for the replications. Correlation studies were performed using CS11 program.

RESULTS AND DISCUSSION

Moisture stress consisted of only one pre-sowing irrigation (Io) had water equivalence of 58.9 and 73.7mm while in restricted moisture regime, one irrigation was applied at 35DAS with water equivalence of 118.9 and 133.7 during 1st and 2nd crop season respectively. Two irrigations applied at 35 and 65DAS comprised normal moisture regime (I_2) had water equivalence 178.9mm in 2009-10 and 193.7mm in 2010-11.

Relative water content

RWC is a measure of plant water status and reflects the metabolic activity in plant tissues (Anjum *et al.,* 2011). Genotypes showed a significant difference in RWC at 65DAS and was highest in MLM-19 (83.6%) and lowest in NLM-80 (67.5%) under moisture stress. NLM-19 possessed highest RWC of 65.6% under restricted moisture. The effect of irrigation and interaction between genotypes x irrigation regimes on RWC were significant only at 120DAS (Table 1). QM-7-335 recorded highest RWC under all moisture regimes, while least was observed in K-9-108 (55.0%) under moisture stress. Genotypes possessed maximum RWC at 65DAS followed by a gradual decline. On an average, RWC was highest (77.4%) in QM-7-335 and least (62.5%) in K-109-113. Under moisture stress (I_0), MLM-19 had highest RWC of 83.6% (65DAS) and 68.6% (120DAS) while QM-7-335 had 73.6% at 90DAS. MLM-19 again registered highest RWC of 84.6% and 75.0% at 65 and 120DAS respectively while QM-7-335 at 90DAS had 78.3% under restricted moisture (I_1). Statistically, RWC did not vary in PLM-2 (90DAS) and NPJ-79 (120DAS) under moisture stress (I_0) and restricted moisture (I_1) regimes. Decline in RWC was 11.5% and 12.5% under stress as compared to normal irrigation module. Water stress was characterized by lower RWC which improved with the increase in soil moisture content as indicated by irrigation levels in the present investigation. Mean RWC was maximum at 65DAS *i.e.* vegetative stage and decreased at later stages of crop growth and development [Table1]. High RWC is a resistant mechanism to water stress which is related to higher osmoregulation. Decrease in RWC under water stress has been reported in oil palm (Sun *et al.,* 2011) and sunflower (Hossain *et al.,* 2011). Recently, similar results have been reported in groundnut by Madhusudan and Sudhakar, 2014).

Water potential (Ψ_w)

Table 1: Relative water content at different growth stages under different moisture regimes.

Genotypes	Relative water content (%)										
	65 DAS			90 DAS				120 DAS			
	Moisture stress(I_o)	Restricted moisture (I_1)	Mean±SE	Moisture stress (I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean±SE	Moisture stress (I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean±SE
K-9-108	65.6	77.3	71.5±5.9	61.0	76.5	78.5	72.0±5.5	55.0	67.1	68.8	63.6±4.3
K-109-113	73.4	78.0	75.7±2.3	64.9	67.0	72.0	68.0±2.1	59.0	60.7	67.7	62.5±2.7
MLM-19	83.6	84.6	84.1±0.5	69.6	70.7	74.9	71.7±1.6	68.6	75.0	77.4	73.7±2.6
NLM-3	78.5	80.1	79.3±0.8	69.5	74.3	78.7	74.2±2.7	63.6	67.2	71.1	67.3±2.2
NLM-80	67.5	73.3	70.4±2.9	63.6	68.8	74.1	68.8±3.0	59.2	59.5	74.9	64.5±5.2
NPJ-79	75.3	76.4	75.9±0.6	66.9	74.0	75.9	72.3±2.7	63.3	63.6	65.1	64.0±0.6
PLM-2	67.8	75.6	71.7±3.9	71.1	71.2	78.2	73.5±2.4	63.4	68.4	68.7	66.8±1.7
PLM-4	70.9	77.9	74.4±3.5	64.4	73.0	74.8	70.7±3.2	64.9	66.4	71.2	67.5±1.9
QM-7-196	75.4	82.3	78.9±3.5	70.8	71.0	73.2	71.7±0.8	62.4	63.9	72.5	66.3±3.1
QM-7-335	74.2	77.6	75.9±1.7	73.6	78.3	80.4	77.4±2.0	67.2	68.3	76.5	70.7±2.9
RLC-1	78.2	79.8	79.0±0.8	67.5	68.3	73.9	69.9±2.0	61.1	63.1	63.4	62.5±0.7
Varuna	74.1	75.8	75.0±0.9	70.9	71.7	72.5	71.7±0.5	62.8	66.1	68.8	65.9±1.7
Mean	73.7	78.2		67.8	72.1	75.6		62.6	65.9	70.4	
CD at 5%	G = 7.81, I = NS, G × I = NS				G = NS, I = NS, G × I = NS				G = NS, I = 3.05, G × I = 10.56		

Table 2: SPAD chlorophyll at different growth stages under different moisture regimes.

Genotypes	SPAD chlorophyll readings										
	65 DAS			90 DAS				120 DAS			
	Moisture stress(I_o)	Restricted moisture (I_1)	Mean±SE	Moisture stress(I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean±SE	Moisture stress(I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean±SE
K-9-108	43.2	44.7	44.0±0.8	42.8	43.3	43.3	43.1±0.2	40.0	40.6	43.6	41.4±1.1
K-109-113	44.2	45.7	45.0±0.8	42.2	44.1	44.4	43.6±0.7	41.0	44.8	45.1	43.6±1.3
MLM-19	43.2	44.8	44.0±0.8	42.4	44.2	44.9	43.8±0.7	43.0	43.6	44.3	43.6±0.4
NLM-3	44.2	47.1	45.7±1.4	42.3	43.5	43.7	43.2±0.4	42.4	44.2	44.5	43.7±0.7
NLM-80	45.6	47.2	46.4±0.8	41.5	45.0	45.9	44.1±1.3	43.9	46.4	47.8	46.0±1.1
NPJ-79	43.2	48.3	45.8±2.6	42.5	43.6	44.1	43.4±0.5	41.3	44.1	48.8	44.7±2.2
PLM-2	43.1	44.3	43.7±0.6	40.9	40.9	41.9	41.2±0.3	40.9	41.0	42.0	41.3±0.4
PLM-4	43.5	45.0	44.3±0.8	42.4	43.5	46.5	44.1±1.2	42.7	42.8	44.1	43.2±0.5
QM-7-196	42.1	44.7	43.4±1.3	39.3	40.5	41.0	40.3±0.5	43.2	43.8	45.5	44.2±0.7
QM-7-335	43.5	46.7	45.1±1.6	38.5	42.0	43.5	41.3±1.5	41.9	46.5	46.5	45.0±1.5
RLC-1	45.1	46.0	45.6±0.5	39.8	39.9	44.7	41.5±1.6	43.8	45.5	51.6	47.0±2.4
Varuna	41.4	41.7	41.6±0.2	38.4	38.6	39.6	38.9±0.4	40.0	40.4	43.6	41.3±1.1
Mean	43.5	45.5		41.1	42.4	43.6		42.0	43.6	45.6	
CD at 5%	G = 2.83, I = NS, G × I = NS				G = 3.18, I = 0.99, G × I = NS				G = NS, I = NS, G × I = NS		

Table 3: Chlorophyll fluorescence parameters under different moisture regimes.

Genotypes	Chlorophyll fluorescence parameters											
	Fo				Fm				Fv			
	Moisture stress (I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean±SE	Moisture stress (I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean±SE	Moisture stress (I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean±SE
K-9-108	71.3	64.8	62.0	66.0±2.8	229.9	255.9	259.9	248.6±9.4	158.6	191.1	197.9	182.5±12.1
K-109-113	66.1	64.7	60.6	63.8±1.7	204.4	250.8	267.6	240.9±18.9	138.3	186.1	207.0	177.1±20.3
MLM-19	77.1	67.3	60.0	68.1±5.0	231.5	240.1	249.7	240.4±5.3	154.4	172.8	189.7	172.3±10.2
NLM-3	72.9	70.3	68.4	70.5±1.3	237.5	245.9	278.8	254.1±12.6	164.6	175.6	210.4	183.5±13.8
NLM-80	72.1	69.7	55.5	65.8±5.2	214.3	240.7	289.3	248.1±22.0	142.2	171.0	233.8	182.3±27.0
NPJ-79	69.5	61.8	61.2	64.2±2.7	227.8	236.9	255.4	240.0±8.1	158.3	175.1	194.2	175.9±10.4
PLM-2	69.2	66.5	60.9	65.5±2.4	215.4	235.7	260.6	237.2±13.1	146.2	169.2	199.7	171.7±15.5
PLM-4	66.1	64.5	62.2	64.3±1.1	216.8	218.2	221.1	218.7±1.3	150.7	153.7	158.9	154.4±2.4
QM-7-196	69.3	67.3	60.5	65.7±2.7	217.1	242.7	246.8	235.5±9.3	147.8	175.4	186.3	169.8±11.5
QM-7-335	75.0	68.0	67.1	70.0±2.0	229.7	236.9	277.3	248.0±14.8	154.7	168.9	210.2	177.9±16.6
RLC-1	67.0	62.8	61.9	63.9±1.6	216.1	233.2	244.2	231.2±8.2	149.1	170.4	182.3	167.3±9.7
Varuna	67.3	63.9	59.8	63.7±2.2	198.4	226.8	235.1	220.1±11.1	149.1	162.9	175.3	156.4±13.2
Mean	70.2	66.0	61.7		219.9	238.7	257.2		149.7	172.7	195.5	
CD at 5%	G = 1.98, I = 1.17, G × I = 4.05				G = 2.81, I = 0.89, G × I = 3.08					G = 3.13, I = 1.55, G × I = 5.35		

Table 4: Chlorophyll fluorescence parameters under different moisture regimes.

Genotypes	Chlorophyll fluorescence Fv/Fo Moisture stress (I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean ± SE	Fv/Fm Moisture stress (I_o)	Restricted moisture (I_1)	Normal moisture (I_2)	Mean ± SE
K-9-108	2.2	2.9	3.2	2.8 ± 0.3	0.718	0.737	0.741	0.732 ± 0.01
K-109-113	2.1	2.9	3.4	2.8 ± 0.4	0.701	0.739	0.741	0.727 ± 0.01
MLM-19	2.0	2.6	3.2	2.6 ± 0.3	0.682	0.714	0.732	0.709 ± 0.01
NLM-3	2.3	2.5	3.1	2.6 ± 0.2	0.681	0.709	0.731	0.707 ± 0.01
NLM-80	2.0	2.5	4.2	2.9 ± 0.7	0.705	0.714	0.726	0.715 ± 0.01
NPJ-79	2.3	2.8	3.2	2.8 ± 0.3	0.723	0.723	0.731	0.708 ± 0.00
PLM-2	2.1	2.5	3.3	2.6 ± 0.3	0.690	0.723	0.731	0.714 ± 0.01
PLM-4	2.3	2.4	2.6	2.4 ± 0.1	0.697	0.698	0.712	0.702 ± 0.00
QM-7-196	2.1	2.6	3.1	2.6 ± 0.3	0.707	0.711	0.721	0.713 ± 0.00
QM-7-335	2.1	2.5	3.1	2.6 ± 0.2	0.703	0.710	0.727	0.713 ± 0.01
RLC-1	2.2	2.7	2.9	2.6 ± 0.3	0.686	0.730	0.731	0.716 ± 0.01
Varuna	1.9	2.5	2.9	2.5 ± 0.3	0.688	0.709	0.714	0.704 ± 0.01
Mean	2.1	2.6	3.2		0.698	0.718	0.728	
CD at 5%	G = 0.11, I = NS, G x I = 0.25				G = NS, I = NS, G x I = NS			

Under stress genotypes of Indian mustard registered lowest water potential which enhanced with the irrigation modules and was highest with the normal moisture regime at 90 and 120DAS. NLM-3 recorded highest water potential (-0.05 MPa) while MLM-19 had comparable Ψ_w of -0.1 MPa at 90 and 120DAS. Ψ_w decreased with increase in water stress at all growth stages (Fig. 2). Our results are in accordance with findings of many workers. Literature cites decline in Ψ_w with the imposition of water stress in crops like *B. juncea* and *B. napus* (Gunasekara *et al.* (2003) and sunflower (Vanaja *et al.,* 2011) and also in soybean (Makbul *et al.,* 2011).

SPAD chlorophyll

Chlorophyll content varied significantly within the cultivars at 65 and 90DAS. SPAD values were highest in NLM-80 (45.6) and NPJ-79 (48.3) and Varuna possessed comparable greenness under I_0 and I_1 respectively at 65DAS. Irrigation modules had significant impact on SPAD values at 90DAS (Table 2). K-9-108 (42.8), NLM-80 (45.0) and PLM-4 (45.9) registered highest SPAD values and Varuna possessed least under all moisture regimes at 90DAS. NLM-80 (Io), QM-7-335 (I_1) and RLC-1 (I_2) were identified having highest SPAD values at 120DAS. Lowest value of SPAD was in cultivar Varuna under stress and restricted moisture regime while PLM-2 had same trait under normal irrigation module. Overall, NLM-80 possessed relatively higher SPAD values under all the three irrigation regimes. Chlorophyll declined under stress by 6.1% at 90DAS and by 8.6% at 120DAS over two irrigations or normal irrigations. (Table 2). Water deficit is known to reduce the chlorophyll content in crop plants as reported by findings of Din *et al.* (2011) and Kauser *et al.* (2006) in *B. napus*. A reduction in chlorophyll a, chlorophyll b and total chlorophyll has been reported in sunflower varieties by Manivannan *et al.* (2007), groundnut (Madhusudan and Sudhakar, 2014) and soybean (Makbul *et al.,* 2011).

Stomatal frequency and size

Cultivars recorded a significant variation in number of stomata per mm² as well as in stomatal size under moisture stress. On abaxial surface, number of stomata per mm² was highest in QM-7-196 (447 ± 11.9), followed by RLC-1 (408 ± 4.6) while least stomatal frequency was registered in K-9-108 (253 ± 1.4) (Fig. 1). On adaxial surface, K-109-113 had highest stomatal frequency of 296 ± 1.4 followed by 283 ± 6.5 while least

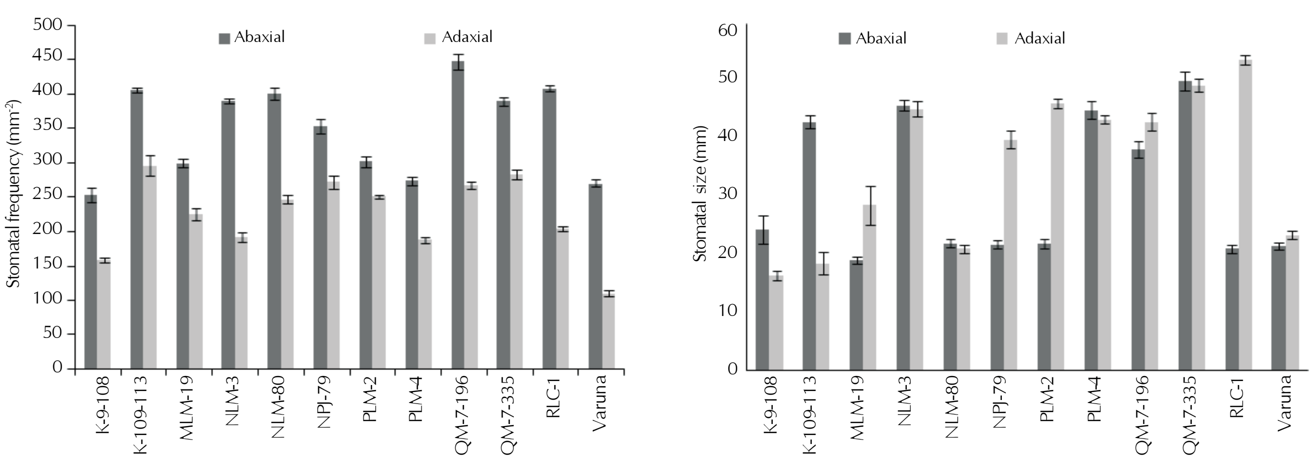

Figure 1: Stomatal characteristics in *Brassica juncea* cultivars on abaxial and adaxial sides at 120 DAS

Table 5: Correlation coefficients for various traits with yield under moisture stress.

	SPAD			RWC			WP			Stomatal characteristics(120 DAS)				Chlorophyll fluorescence parameters (90 DAS)						Drought susceptibility index			Drought tolerance index		
	65 DAS	90 DAS	120 DAS	65 DAS	90 DAS	120 DAS	65 DAS	90 DAS	120 DAS	SF (aba)	SS (ada)	SF (aba)	SS (ada)	Fo	Fm	Fv	Fv/Fo	Fv/Fm	DSI1	DTI1	DSI2	DTI2	DSI3	DTI3	Yield
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1																								
2	.491	1																							
3	636*	.727**	1																						
4	.332	-.052	.091	1																					
5	.004	-.267	-.335	.234	1																				
6	-.097	.077	-.065	.091	.234	1																			
7	-.069	-.428	-.246	.143	.216	.088	1																		
8	-.101	-.638*	-.233	.318	.326	-.270	.348	1																	
9	.375	.034	.274	.366	.320	.163	.568	-.026	1																
10	.282	.225	-.021	.437	.030	-.018	.428	-.268	.575	1															
11	.045	.176	.201	.214	-.498	.244	.038	-.072	.066	.346	1														
12	.065	.166	.166	-.647*	-.208	-.269	.305	.056	.284	.648*	.287	1													
13	.329	.564	.534	-.057	-.100	-.111	.062	-.330	.399	.324	.283	.216	1												
14	.315	.288	.418	.210	.094	.066	.076	.083	.201	.007	-.030	.149	-.021	1											
15	.272	.048	.271	.200	.147	-.214	.173	.399	.186	.009	.164	.125	.305	.724**	1										
16	.218	-.049	.179	.169	.146	-.289	.185	.463	.155	.009	.214	.099	.385	.520	.966**	1									
17	-.013	-.302	-.073	.040	.000	-.403	.265	.505	.085	.062	.360	.094	.468	-.187	.528	.723**	1								
18	-.512	-.444	-.459	.060	-.243	-.568	-.067	.193	-.260	.043	-.009	.322	-.262	-.105	.086	.146	.253	1							
19	.431	-.225	.124	.278	.459	.329	.500	.200	.685*	.045	-.282	-.163	.011	.316	.231	.168	-.062	-.424	1						
20	-.343	.134	.033	.043	-.010	.023	-.454	.179	-.479	-.268	.207	.059	-.293	.328	.203	.128	-.116	.194	-.586*	1					
21	.323	-.154	-.157	-.160	.498	.431	.122	.036	.157	-.182	-.278	-.696*	-.204	.261	.194	.142	-.137	-.478	.652*	-.290	1				
22	.310	-.269	-.136	.111	-.164	-.035	.161	.010	.308	.317	.333	-.127	-.127	.094	.216	.233	.202	.220	.315	-.334	.398	1			
23	-.192	-.249	.177	.235	-.490	-.208	.222	.194	.300	.152	.453	.472	.090	-.227	-.112	-.053	.269	.378	-.080	-.062	-.640*	.166	1		
24	-.032	.015	-.068	.179	.623*	.410	.415	.027	.480	.193	-.058	.033	.503	.139	.354	.386	.283	-.308	.458	-.234	.285	-.202	-.261	1	
25	-.319	.318	-.234	-.213	.265	.103	-.261	-.226	-.416	.010	-.001	-.049	.265	-.044	.107	.149	.106	-.015	-.525	.424	-.077	-.501	.574	.400	1

*indicates correlation coefficients significant at 5%, ** significant at 1%

Table 6: Correlation coefficients for various traits with yield under restricted moisture

SPAD	RWC			WP			Chlorophyll fluorescence parameters							Drought susceptibility/tolerance indices						
	65	90	120	65	90	120	65	90	120	Fo	Fm	Fv	Fv/Fo	Fv/Fm	DSI1	DTI1	DSI2	DTI2	DSI3	DTI3	Yield
	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	1																				
2	-.055	1																			
3	.540	.430	1																		
4	.520	-.405	-.009	1																	
5	.110	-.083	.189	.040	1																
6	-.568	.394	-.363	-.510	-.387	1															
7	-.084	.130	-.316	-.098	-.133	.622*	1														
8	-.019	.025	-.038	.069	.338	.258	.612*	1													
9	-.081	-.166	-.364	.056	-.387	.237	.743**	.361	1												
10	.116	.112	.167	-.349	.494	-.254	.180	.319	.318	1											
11	.144	.033	-.101	.348	.419	-.100	.252	.375	.083	.287	1										
12	.118	.003	-.151	459	.302	-.035	.214	.304	-.000	.026	.965**	1									
13	.067	-.123	-.265	.635*	-.046	.118	.179	.199	-.038	-.493	.675*	.840**	1								
14	-.103	-.266	-.284	.477	-.041	.085	.105	.082	-.055	-.391	.662*	.798**	.891**	1							
15	.489	-.336	.230	.228	.005	-.154	.088	.230	.325	.136	-.037	-.076	-.054	-.026	1						
16	-.139	.422	.284	-.207	.667*	-.220	-.149	.224	-.415	.475	.312	.196	-.155	-.191	-.586*	1					
17	.366	-.044	.273	-.066	.147	-.126	-.257	-.104	-.137	.154	.177	.143	.022	.030	.652*	-.290	1				
18	.296	.156	-.235	.164	-.254	.213	.282	.203	.427	.007	.161	.166	.212	-.111	.315	-.334	.398	1			
19	-.188	-.033	-.318	.061	-.251	.244	.368	.453	.414	-.075	-.372	-.368	-.168	.315	-.080	-.062	-.640*	.166	1		
20	.265	-.060	.143	-.099	.152	-.057	.127	-.102	.110	.293	.118	.043	-.144	.105	.458	-.234	.285	-.202	-.261	1	
21	.295	-.022	.272	-.095	.250	-.169	-.087	-.297	-.130	.259	.301	.244	-.002	.253	.371	-.127	.538	-.231	-.716**	.861**	1

*indicates correlation coefficients significant at 5%, ** significant at 1%

frequency of 111±4.6 in Varuna. On abaxial/upper surface, QM-7-335 possessed maximum stomatal size of 50.1±1.7 mm, followed by 45.9 mm in NLM-3 and minimum stomatal size of 19.0±0.6 mm in MLM-19. Mean stomatal frequency was 349.3 mm^2 and size 31.2 mm on abaxial side. On adaxial side, maximum stomatal size was in RLC-1 (53.8±0.8 mm), followed by 49.2±1.2 mm in QM-7-335 while minimum stomatal size (16.4±0.9) was in K-9-108) (Fig. 1). Plants are known to have lower stomatal frequency under normal moisture conditions as compared to that under water stress. Stomatal frequency in the *B. juncea* genotypes was higher on abaxial than adaxial surface which is in accordance with the results of Nerkar *et al.* (1981) in *Vicia faba*. However, Maghsoudi and Maghsoudi (2008) reported higher stomatal frequency on on abaxial surface in wheat cultivras under drought stress.

Chlorophyll fluorescence

Genotypes exhibited a significant difference in the all the chlorophyll fluorescence parameters except quantum yield of PSII (Fv/Fm) as evident from Table 3 and 4. Non significant difference were accorded to water splitting capacity on the donor side of PSII (Fv/Fo) and status of PSII (Fv/Fm) with irrigation modules only however interactions were non significant only for Fv/Fm. Most of the fluorescence parameters showed significant variations.

Highest initial fluorescence (71.3) was recorded in MLM-19 while minimum Fo (66.1) was in K-109-113 and PLM-4 under moisture stress. NLM-3 and NPJ-79 registered highest (70.3) and lowest (61.8) Fo respectively under one irrigation regime. Similarly, under normal moisture also NLM-3 (68.4) had highest and NLM-80 (55.5) the lowest Fo values. Araus *et al.,* (1998) observed highest F_0 values in stressful conditions. Maximal fluorescence (Fm) and variable fluorescence (Fv) were highest again in NLM-3 whereas lowest in Varuna under moisture stress. Maximum Fm and Fv under restricted moisture were in cultivar K-9-108 while PLM-4 had the least values for these two parameters. Again under two irrigations (I_2), PLM-4 had the least values of Fv and Fm while highest was in NLM-80 (Table 3). Overall, the data indicated highest value of Fo under stress which decreased with irrigations. Mean initial fluorescence was 12.1% higher under stress over normal moisture regime. Fo values are related to chlorophyll fluorescence of PSI receptors and considering significant Fo differences between the cultivars, it seems the receptors chlorophylls had variable efficiency. As SPAD values decreased with moisture stress it should be partly responsible for photo inhibition. Under drought stress, recovery of material especially nitrogen will interrupt and furthermore, chloroplasts needs N to generate chlorophyll through proteins and under nitrogen or water limited condition, chlorophyll production rates became slower and as a result leaves will become more susceptible to photo inhibition (Sharma, 2014). On the other hand, Fm and

Table 7: Correlation coefficients of various traits with yield under normal moisture

	SPAD		RWC		WP		Chlorophyll fluorescence parameters					Drought susceptibility/tolerance indices						
	90 DAS	120 DAS	90 DAS	120 DAS	90 DAS	120 DAS	Fo	Fm	Fv	Fv/Fo	Fv/Fm	DSI1	DTI1	DSI2	DTI2	DSI3	DTI3	Yield
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1																	
2	.232	1																
3	.033	-.529	1															
4	.168	-.578*	.919**	1														
5	.032	.138	.226	.205	1													
6	-.205	-.324	.466	.369	.371	1												
7	.673*	.024	.132	.144	-.052	-.085	1											
8	.416	.300	.107	.125	.145	.118	.124	1										
9	.302	.298	.085	.101	.155	.134	-.049	.985**	1									
10	-.051	.276	.018	.024	.201	.131	-.524	.775**	.871**	1								
11	.265	-.206	-.196	-.015	.178	.087	.101	.542	.528	.377	1							
12	-.191	.233	-.297	-.298	.564	.369	-.183	-.094	-.063	.049	.081	1						
13	.399	.429	-.266	-.178	-.365	-.797**	.113	.302	.285	.221	.033	-.586*	1					
14	-.049	.047	-.273	-.135	-.002	.055	.081	-.063	-.077	-.113	.092	.652*	-.290	1				
15	-.186	.109	.111	.175	.259	.452	.191	-.004	-.037	-.123	-.064	.315	-.334	.398	1			
16	-.168	.195	.318	.191	.585*	.154	-.141	-.117	-.094	.027	-.325	-.080	-.062	-.640*	.166	1		
17	.346	-.024	-.016	.040	.187	.137	.187	.105	.073	-.014	.209	.458	-.234	.285	-.202	-.261	1	
18	.197	.138	.206	.170	.587*	.201	.075	-.015	-.028	-.080	.364	-.241	-.179	-.058	-.483	.483	.717**	1

*indicates correlation coefficients significant at 5%, ** significant at 1%

Fv values were lower by 16.9% and 30.5% respectively under water deficit and increased with irrigations. Nevertheless, when the fluorescence value of chlorophyll a is low, electron acceptor Q is in oxidation state and as a result Fv decreased. Further, Q in oxidation state under drought stress reveals disruption in normal electron transfer in photolysis of water at PSII. Although, water limited condition caused to quantum efficiency of net photosynthesis declined. Environmental stresses reduce Fv via inhibition of PSII photo oxidation. Since, Fv with irrigation modules increases indicating full reduction of electron acceptor (Q) hence no disruption of electron transfer to PSI and also high Fm values in the present study. Further, it may be accepted that drought stress has disturbed electron transfer to PSI (Paknejad *et al.*, 2007).

The efficiency of water splitting complex on the donor side of PSII (Fv/Fo) is the most sensitive component of the photosynthetic electron transport chain. Decrease in this ratio results from electron transport impairment. Further an inhibition of osmotic ally driven uptake of water is also observed under moisture deficit inferred by lower Fm values which indicates the accumulation of inactive PSII reaction centre and may also be due to D1 degradation (Kalaji *et al.*, 2011). Highest ratio of Fv/Fo was recorded in NLM-3, NPJ-79 and PLM-4 with water deficit (I_o), K-9-108 and K-9-113 with one irrigation (I_1) and NLM-80 with two irrigations (I_2). Under stress the decline in mean Fv/Fo ratio was 52.3% over normal moisture regime (Table 4). Disruption in photochemical efficiency of PSII was to the tune of 4.3% under stress. The Fv/Fm values in NPJ-79 under stress and restricted moisture was only1.1% than that noted in control plants (I_2) indicating reduced moisture damaged the reaction centers and also reducing electron transport capacity in PSII. Similarly in cultivar PLM-4 disruption of PSII was higher *i.e.* 2.1% over normal moisture regime. Rest of the genotypes exhibited variable damage of PSII under water stress (Table 4). Pospisil *et al.* (1998) stated that environmental stresses like water deficit affects the PSII efficiency and therefore reduced the maximum quantum yield of PSII (Fv/Fm). Literature cites that under limited moisture, Fo increased and Fm decreased. A reduction in PSII quantum yield has been reported in *Phaseolus vulgaris* (Ghanbari *et al.*, 2013)), *B. napus* (Kauser *et al.*, 2006).

Correlation studies

Association between different parameters under moisture stress is evident from Table 5. SPAD values at 120DAS had significant positive association with chlorophyll at 65DAS (r=0.636*) and also at 90DAS (r=0.727**). Water potential and SPAD at 90 days after sowing (r=-0.638*) had negative correlation. However, stomatal frequency on abaxial surface and water potential exhibited positive relation (r=0.575*) recorded 120DAS. Stomatal frequency on adaxial and abaxial sides had positive association (r=.648*). RWC at 90DAS had significant positive correlation with DTI3 (r=.623*). SPAD at 120DAS had a positive correlation with stomatal frequency on abaxial side (r=.575) and DSI1 (r=.685*). Stomatal frequency on adaxial side was found to be negatively correlated with DSI2 (r=-.696*). Among the chlorophyll fluorescence parameters, highly positive significant correlations were observed between Fo and Fm (r=.724**), Fm and Fv (r=.966**) and Fv/Fo and Fv (r=.723**). DSI1 had a negative

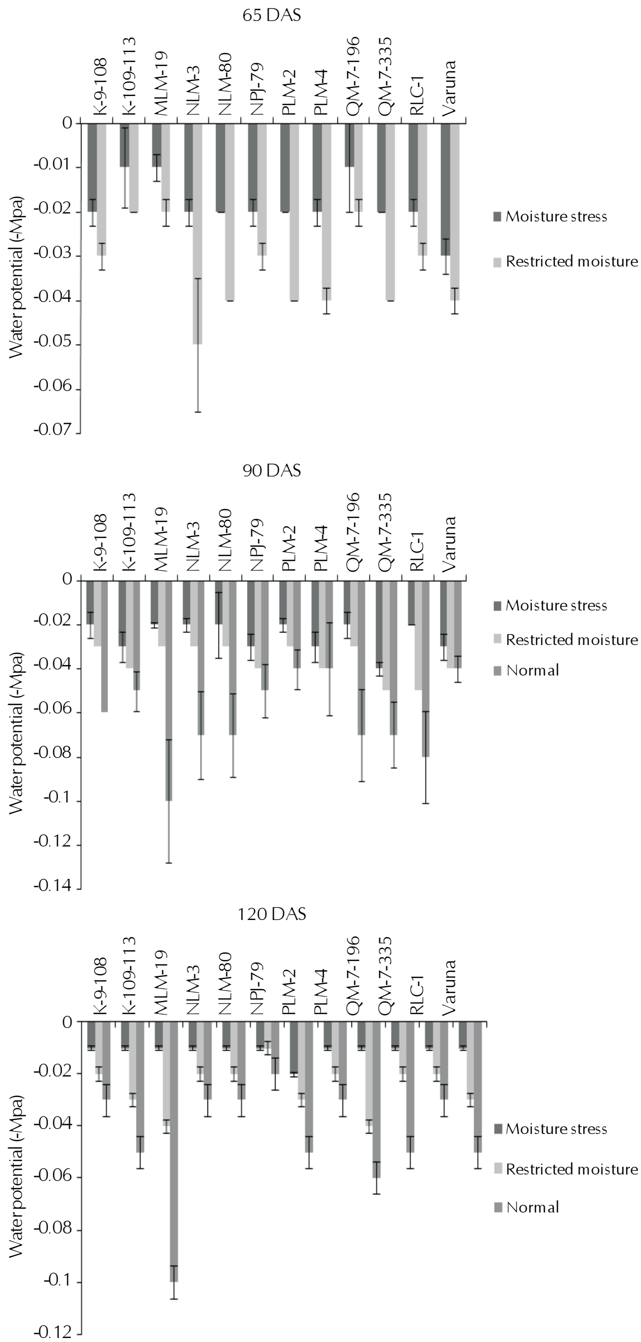

Figure 2: Water potential in *Brassica juncea* cultivars at different growth stages

correlation with DTI1 (r=-.586*) but was positively correlated with DSI2 (r=.652*). DSI2 and DSI3 were negatively correlated (r=-.660*) (Table 5). SY had positive association with SPAD (r=0.318) and RWC (r=0.265). At 90DAS, stomatal size on adaxial surface (r=0.265), Fv/Fo (r=0.106) and DTI1 (r=0.429), DSI3 (r=0.574) and DTI3 r=(0.400). Weak negative correlation existed between SY and Fv/Fm (r=-0.015) and DSI1 (r=-0.077) but high magnitude negative association existed for DTI2 and SY (-0.501). Physiological parameters exhibited significant correlations under restricted moisture (Table6) too. Ψ_w at 65DAS had a positive correlation with Fv/Fo (r=.635*). SPAD chlorophyll at 65 DAS recorded a significant positive correlation with SPAD at 90DAS (r=.612*), 120DAS (r=.743**) and Ψ_w at 120DAS (r=.622*). A good deal of significant positive correlations were observed within the chlorophyll fluorescence parameters. Fm had a positive correlation with Fv (r=.965**), Fv/Fo (r=.840**) and Fv/Fm (r= .662*). Fv was positively correlated with Fv/Fo (r=.891**) and Fv/Fm (r=.798**). DSI1 and DTI1 were negatively correlated (r=-.586*), Similarly, significant negative correlations were observed between DSI2 and DTI2 (r=-.640*). DSI3 was negatively correlated with the yield (r=-.716**) and significant positive correlation was found between DTI3 and yield (r=.861**). SY had positive correlation with SPAD (r=0.295) at 65DAS and (r=0.272) at 120DAS, with RWC at 90DAS (r=0.250), Fo(r=0.301), Fv(r=0.244), Fv/Fm(r=0.253). SY showed highly negative association with DSI3 (r=-0.716**) and DTI3 (r=-0.861**).

Significant correlations were observed among various traits under two irrigation module (Table7). At 90DAS, RWC was positively correlated with Fo (r=.673*). At 120DAS, Ψ_w was negatively correlated with RWC (r=-.578*) and positively correlated with Ψ_w at 90 DAS (r=.919**). At same stage of crop growth SPAD chlorophyll values had positive correlation with DSI3 (r=.585*) and yield (r=.587*). However, SPAD at 120 DAS was negatively correlated with DTI1 (r=-.797**). Highly significant positive correlations were observed between Fm and Fv (r=.985**), Fm and Fv/Fo (r=.775*) and Fv and Fv/Fo (r=.871**). DTI1 was positively correlated DTI2 (r=.652*) and negatively correlated with DTI1 (r=-.640*) and DTI3 (r=-.640*). DTI2 was positively correlated with yield (r=717**). SY had positive association with SPAD and RWC except RWC at 90DAS, Fo r=(0.75), Fv/Fm (0.364), DSI3 (r=0.483) and DTI3 (r=0.717**).

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