Assessment of drought tolerance of two cultivars of Indian mustard (*Brassica juncea*) using morphological and physiological parameters

ABSTRACT

Aims: Drought stress may affect the Morphology, biochemistry, growth, and physiology of *Brassica juncea* (Indian mustard), a low-cost, oil-yielding, and economically significant plant. In this paper assessing various growth, morphological, and physiological parameters under different irrigation regimes could offer some significant information for the selection of a suitable and appropriate genotype for breeding.

Study design:Two commonly grown cultivars (RH-725 and RH-749) of mustard in Haryana under four irrigation regimes were evaluated. The applied Irrigation regimes were: control (double irrigation: once at the 50% flowering and another at the 50% fruiting stages), early irrigation (at 50% flowering only), late irrigation (at 50% fruiting only), and stress (no irrigation). The plants were exposed to short-term water shortages during the vegetative and reproductive growth stages. Drought stress in both stages had a negative effect on the morphological and physiological parameters of mustard.

Place and Duration of Study: An open field experiment was performed at the Nursery of Kurukshetra University, Kurukshetra, Haryana, India, located at the latitude of 290–95° north and longitude of 760–82° east, with a height of 258.4 m above sea level. Crop sown under field conditions from October to March for two consecutive years (2018-2019, 2019-2020) at a temperature of 30-32 °C (days) and 15-25 °C (nights)

Methodology: Measured adaptability using 15 morphological, 4 physiological, and 7 different growth parameters. PCA data revealed that physiological and growth parameters are more sensitive than morphological parameters in distinguishing the control and drought treatments. Alterations in physiological parameters occurred at all three levels of water stress. Growth analysis and yield parameters decreased with the severity of stress. Drought-stress treatments gradually reduced the Morphological and physiological traits of mustard.

Results: The study revealed a relationship between *Brassica* species' adaptation to drought stress and the rapidity, severity, and duration of the drought event. This highlights the importance of considering these factors while selecting genotypes for breeding programmes aimed at improving drought tolerance in *B. juncea*. Overall, the research provides valuable information for selecting suitable and appropriate genotypes for breeding drought-tolerant varieties of *B. juncea*. By understanding the specific physiological (relative water content, leaf osmotic potential, membrane stability index, electrolytic index)and growth parameters (plant height, root length, fresh and dry weight of leaves and roots, leaf area etc.) that are more sensitive to drought stress. Furthermore, this study revealed a relationship between Brassica species' adaptation to drought stress and the rapidity, severity, and duration of the drought event. Based on PCA values, the cultivar RH-749 performed better both morphologically and physiologically under all stress conditions compared to RH-725. This suggests that RH-749 is more adapted and tolerant to drought stress, making it a potential candidate for breeding and cultivation in water-limited environments

***Key words**: -Brassica juncea; Drought; Irrigation regimes; Plant productivity; Growth; Morphology; Physiology.*

1. INTRODUCTION

Climate change, combined with the resulting desertification and overexploitation of water resources, due to overpopulation and intensification of agriculture, will be a challenge for the survival, growth, and sufficient yield of agricultural commodities (Passioura, 2007). Drought is widely acknowledged as one of the most limiting abiotic stresses affecting global agricultural productivity. Scarcity of water is a major environmental constraint to plant survival, growth and productivity (Farooq et al. 2009). Crops experience drought stress due to imbalances between water supply to the roots and transpiration rate needs. Generally, drought stress conditions elicit a cascade of morphological, physiological, biochemical and molecular responses that affect plant metabolism (Osakabe et al. 2014). Among others, stomatal closure is induced, causing a decrease in carbon dioxide uptake followed by a reduction in photosynthetic activity (Dubey et al. 2001). Moreover, drought causes nutrition disturbances as roots are unable to take up a range of nutrients from the soil due to reduced root activity, slow ion diffusion and water movement rates, and disruption of water continuity in soil pores (Gomez et al. 2012). To overcome the consequences of water deficit, plants evolved different kinds of adaptive strategies that include stress avoidance and stress tolerance mechanisms (Fang and Xiong 2015).

Brassica juncea is generally grown under diverse agro-climatic conditions in India and belongs to the Brassicaceae (Cruciferae) family of angiosperm (Shakeel et al. 2019). Drought affects the growth and development of plant by altering their morphological, physiological, biochemical attributes of plant. *Brassica juncea* (L.) CzernandCoss., vernacularly known as Indian mustard, is an annual herbaceous plant that is grown in the winter (rabi) season and generally requires a good supply of water during the growing and harvesting periods. In India, *Brassica juncea* is a low-cost oil crop.Indian mustard is an allopolyploid (AABB, 2n = 36) member of the Brassicaceae family that was created through natural hybridization of two Brassica species, *Brassica rapa* (AA,2n=20) and *Brassica nigra* (BB, 2n = 16), followed by spontaneous chromosome doubling (Jiang et al. 2015; Yang et al. 2016).   Indian mustard is used as the source of vegetables, oilseed, forage and fodder, green manure, condiments and occupied a unique position in world agriculture (Singh et al. 2018)

Restrictive irrigation, also known as supplementary irrigation or limited irrigation, is an irrigation method by which the actual evapotranspiration of crops is less than the potential evapotranspiration or where the amount of irrigation cannot fully meet the crop water demand (Wang 2017; Thapa et al. 2020; Shivran et al. 2019). In this study, morphological and physiological traits of the crop were compared between two cultivars of *Brassica juncea* with four different irrigation regimes. The objective of the study was to observe the responses of two mustard cultivars differing in their relative tolerance to drought.

2. MATERIAL AND METHODS

2.1 Experimental site and location

To evaluate the performance of *B. juncea*, an open field experiment was performed at the Nursery of Kurukshetra University, Kurukshetra, Haryana, India, located at the latitude of 290–95° north and longitude of 760–82° east, with a height of 258.4 m above sea level. Crop sown under field conditions from October to March for two consecutive years (2018-2019, 2019-2020) at a temperature of 30-32 °C (days) and 15-25 °C (nights). The topography was nearly homogeneous, with a sandy clay loam texture. The experimental field has a mild slant that allows excess water to drain freely, which is critical for growing *Brassica* crops.

2.2 Study material

The present investigation was conducted on two cultivars of *Brassica juncea*, RH-725 (Rainfed) and RH-749 (irrigated). Certified seeds of *B. juncea*were procured from the oil seed section of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana.

Figure 1. Experimental field and crop with different water treatments

2.3 Experiment design and treatment

Plants were grown in open field conditions. *B.juncea* seeds of the RH-725 and RH-749 cultivars were sown during the 2^{nd} week of October each year. The experimental layout was a Randomised Complete Design with three replicates. The number of replications was kept at three for all observations. The size of the block was 6 X 3 feet, with 5 to 6 rows of plants, with a 30 cm distance between each plant. Fertilisers were applied on the 7th day after sowing in the field. Water stress was created by withholding irrigation at the initiation of the flowering stage and the siliquae formation stage. For irrigation in the field, proper channels were prepared. Plant protection was given to all the treated plants, *i.e.,* hoeing, trellising, thinning, and weeding were carried out throughout the growing season.

Water stress was created by withholding irrigation during the critical growth stages (Flower initation and siliquae development) of the crop. Irrigation regimes were

(1) Control (two-time irrigation)
(2) Early irrigation (irrigation at flower initiation)
(3) Late irrigation (irrigation at siliquae development)
(4) No irrigation (stress)

At the vegetative, flowering, and siliquae development stages, sampling was carried out. Every time, three replicates of each treatment were sampled, along with a control, to record the different metrics at the sampling stages. Leaves, roots, and whole plants of each cultivar were oven-dried for 48 hours at 60°C, then for 24 hours at 80 °C, or until a constant dry weight were achieved. It was recorded in grammes. Plant height was measured using a metre scale from the soil surface to the apex of the plant, i.e., main shoot length, and expressed in centimetres. The leaf area per plant (sq. cm.) was estimated using a portable leaf area metre (Systronics 211, Ahamadabad, India) according to the manufacturer's instructions. Using the formula provided by (Sestak et al. 1971), calculate LAI, CGR, RGR, and NAR. Using the (Weatherley 1950), formula, the relative water content was calculated and the osmotic potential was determined by the Model 5100-B Vapour Pressure Osmometer, Wescor Inc., Logan, Utah, USA. On the basis of electrolytic leakage in leaves, the membrane stability index (MSI) and electrolyte leakage (EI) were determined by using the formulas given by (Deshmukh et al. 1991; Lutts et al. 1996), respectively.

2.4 Statistical analysis

The data was statistically analysed using an online Statistical Analysis tool (OPSTAT, CCS Haryana Agricultural University, Hisar). Three factorial Randomised block designs (RBDs) were used in the investigation. The number of replications was kept at three for all observations. The critical difference at a 5% level of significance was used to determine the relevance of the data received. XLSTAT statistical software was used to determine the PCA, and cluster analysis was performed using the Ward technique by the PAST statistics programme to better understand the variation patterns among the accessions (Hammer et al. 2001). Heat map matrices are also performed by SR Plot statistical software (Lumivero 2023).

3. Resultsand Discussion

*B.Juncea*has a diverse geographic distribution. In this study, we used 26 various parameters to assess drought tolerance in cultivars. Decreasing irrigation water significantly decreased plant height, root length, dry and fresh weight of the plant, leaf area, number of primary and secondary branches, number of siliquae per plant, seed yield, and oil content of the crop (Table 1). Control irrigation resulted in the highest values of these growth parameters, followed by early irrigation, late irrigation, and stress. Increasing water stress reduced growth characteristics, which may be due to a reduction in photosynthesis and plant biomass. Reduced stomatal and mesophyll conductance resulting from increased water stress levels led to low CO_2 availability, which in turn hindered photosynthesis.

Increased irrigation has a noticeable impact on growth, which may be explained by the root system having access to enough moisture.

Usually, fluctuations in plant height are the most conspicuous characteristic of genetic conditions and environmental changes in most plants. Applying water deficit stress caused a reduction in the plant's height. This reduction in plant height due to water deficit stress is probably related to a decline in photosynthetic products as a result of soil moisture decrease, which eventually causes the plant to not reach its genetic potential. Cell division, expansion, and elongation are all harmed by extreme water stress, leading to decreased crop growth and production. Water shortages slowed plant growth, while water stress shortened stem length in soybeans and rice. (Sadaqat et al. 2003), have also reported a significant decrease in the stem height of rapeseed cultivars under water stress conditions.

The occurrence of drought stress during the flowering and fruiting stages increased siliquae seed lessness due to insufficient fertility and flower abscission; as a result, it reduced the number of seeds per silique (Wright et al. 1996). Due to a significant lack of water, disruption of a water passage from the xylem to the fencing elongating cells inhibits cell elongation in higher plants. Root-to-shoot transmission is disrupted, resulting in stunted shoot and root growth, reduced photosynthetic machinery, oxidation of chloroplast lipids, early leaf senescence, and changes in pigment and protein structure. In rapeseed, it has been reported that plant height, number of branches, and number of siliquae per plant, as well as seed yield, decreased significantly under water stress conditions (Bitarafan and Shirani-Rad 2012; Moosavia et al. 2014; Nazemi and Alhani 2014; Rad et al. 2014a; Rad et al. 2014b; Zakerin et al. 2014).

Table 1. Morphological traits in two brassica cultivars: RH-725 and RH-749 with four irrigation regimes

Traits	**Control irrigation**		**Early irrigation**		**Late irrigation**		**Stress**	
	RH-725	**RH-749**	**RH-725**	**RH-749**	**RH-725**	**RH-749**	**RH-725**	**RH-49**
Plant height (cm)	98.748	104.748	94.935	98.578	86.628	88.757	73.738	78.746
Root length (cm)	19.046	19.364	18.337	18.693	16.393	16.758	13.345	13.794
Fresh weight of leaves (g)	9.679	9.817	9.132	9.756	7.369	7.876	7.236	7.679
Dry weight of leaves (g)	0.445	0.455	0.435	0.441	0.334	0.343	0.252	0.302
fresh weight of root (g)	12.29	12.87	10.18	10.67	9.162	9.742	7.769	7.982
dry weight of root (g)	4.973	4.988	4.675	4.894	3.674	3.895	3.256	3.786
Leaf area	92.43	95	95.65	98	82.65	87	71.65	72
Number of leaves per plant	10.64	10.98	10.23	11.54	10.65	11.12	8.69	9.64

Number of primary branches	7.02	7.63	6.23	6.55	5.19	5.26	4.56	4.86
Number of secondary branches	10.23	11.03	9.54	9.88	7.84	7.96	6.32	6.96
Siliquae per plant	245	251	235	242	229	234	199	210
Siliquae length	4.63	5.40	4.30	4.50	4.83	5.07	8.10	8.07
Number of seeds per siliques	7	7	8	9	8	9	6	7
1000 seed weight	4.206	4.384	4.184	4.254	4.021	4.147	3.648	3.759
Oil content	37.15	37.25	37.10	37.15	36.54	36.66	35.25	35.32

From the observed value of relative water content, it was found that RWC sharply declined during the flowering and fruiting stages in comparison to the vegetative stage. Cultivar RH-749 maintained higher relative water content as compared to another cultivar (Table 2). Relative water content (RWC) of leaves decreases gradually with the age of plants in both cultivars. Revealing the plants to low soil moisture content by maintaining irrigations caused a significant decrement in the RWC of all leaves at all growth stages, and the osmotic potential of sample leaves diminished (more negative) with successive growth stages. Leaf water potential was highest (less negative) in two irrigation treatments for both cultivars of *B. juncea*. Drought stress reduced the number of leaves per plant and the size of the leaves, which is mostly dependent on the water balance or turgor pressure of the leaves.

The membrane stability index, which is proportional to cell membrane damage, is a physiological metric commonly used to assess drought resistance. Cell membrane stability is an established index to evaluate crop plants against abiotic stress tolerance (Kumar et al. 2016). The membrane stability index decreased from vegetative to further developmental stages, decreasing progressively with the age of the plant and then sharply declining at the fruiting stage. Abiotic stresses frequently target biological membranes first and can impair the electron transport chain (ETC) and enhance the degree of membrane fatty acid saturation by changing the properties of proteins, ultimately increasing the permeability of the plasma membrane. It is commonly acknowledged that electron transport in chloroplasts, mitochondria, peroxisomes, and plasma membranes increases reactive oxygen species (Pooja et al. 2019; 2020). Electrolyte leakage was increased in both cultivars under water stress conditions. A significant decline in MSI under-withholding water irrigation treatments was observed in both cultivars. Cultivar RH-749 had more MSI thanRH-725 at all sampling stages.

The result of leaf area shows that (Table 2), leaf area increased progressively from vegetative to fruiting stages. Maximum leaf area was found in control irrigation (185 sq. cm) in cultivar RH-749 at the fruiting stage, followed by 181 sq. cm in RH-725. The number of primary branches in both cultivars increased with time. A higher number of branches were recorded at the fruiting stage in *cv*. RH 749. Cultivar RH-749 showed a greater number of primary branches in comparison to cultivar RH-725. Statistically, significant variation was recorded for secondary branches of the stem of mustard at all stages in both cultivars. The cultivar RH-749 produced significantly more siliquae than RH-725. A maximum number of siliquae was recorded for plants that were irrigated twice (control), then single irrigation, and minimum siliquae were recorded from no irrigation water treatment. Siliquae length was also significantly affected by water stress; maximum siliquae length was observed in two irrigation water treatments in both cultivars. No irrigation and single irrigation resulted in a distinct decrease in the number of seeds per siliquae in both cultivars (Table 3). The decrease in the number of seeds was significantly higher in the RH-725 cultivar as compared to the RH-749 cultivar. Maximum seed number per siliquae was observed in (9) RH-749 cultivar, which is followed by (8) RH-725 cultivar under control irrigation.

1000 seed weight results revealed that the cultivar RH-749 had a significantly higher 1000 seed weight than the RH-725 cultivar. Low soil moisture resulted in a significant reduction in seed weight in both cultivars. A decrease in seed yield under stress conditions was observed in both cultivars. Water stress resulted in a marked decrease in oil content in both cultivars, and the decrease in oil content was considerably higher in RH-725 than in RH-749. The maximum oil content was observed in two irrigation water treatments. Among early and late irrigation water treatments, there was more oil content in early irrigation as compared to late irrigation.

Our findings show that different irrigation regimes vary the values of LAI, CGR, RGR, and NAR in *B. juncea*, which could indicate greater carbohydrate utilisation and photo-assimilate transport. Under such conditions, the cells generated are large and have thin walls, potentially increasing the leaf area index. The LAI of a crop at a given growth stage represents the crop's photosynthetic potential or dry matter accumulation level. With the steady increase in plant development under varied treatments, LAI rose due to an increase in dry matter accumulation. Adequate moisture availability at important growth stages (flower initiation and siliquae development) may have allowed the plants to achieve higher biomass, resulting in a higher crop yield.

Table 2. Physiological traits in two brassica cultivars: RH-725 and RH-749

Traits	**Control irrigation**		**Early irrigation**		**late irrigation**		**Stress**	
	RH-725	**RH-749**	**RH-725**	**RH-749**	**RH-725**	**RH-749**	**RH-725**	**RH-49**
Relative water content (RWC)	77.23	77.52	72.71	73.25	74.14	74.56	69.28	70.19
Leaf Osmotic potential	0.684	0.578	0.738	0.675	0.835	0.758	1.045	0.985
Membrane stability index (MSI)	65.61	68.17	63.19	66.16	60.53	63.71	54.78	58.29
Electrolyte leakage	25.63	23.52	28.76	27.69	32.18	30.48	40.34	35.73

Table 3. Growth analysis traits in two cultivars: RH-725 and RH-749

Traits	**Control irrigation**		**Early irrigation**		**late irrigation**		**Stress**	
	RH-725	**RH-749**	**RH-725**	**RH-749**	**RH-725**	**RH-749**	**RH-725**	**RH-49**
Leaf area index (LAI)	0.306	0.317	0.316	0.327	0.272	0.290	0.237	0.240

Crop growth rate (CGR)	0.343	0.353	0.329	0.334	0.244	0.247	0.212	0.223
Relative growth rate (RGR)	0.012	0.013	0.011	0.012	0.009	0.010	0.007	0.007
Net assimilatory rate (NAR)	0.105	0.056	0.104	0.055	0.066	0.036	0.050	0.027
Seed yield	1634	1764	1587	1624	1468	1569	1264	1364
Biological yield	4587	4965	4123	4269	3648	3794	3125	3026
Harvest index	35.62	35.53	38.49	38.04	40.24	41.35	40.45	45.08

3.1 Evaluation of the drought responses in cultivars by PCA, Heat map and agglomerative clustering methods

Main aim of PCA to determine the number of main factors that can be extracted to decreases the number of effective parameters. Two cultivars of *B.juncea* (L.) morphological, physiological, and growth index parameters were used for the calculation of Eigen value, Variability percentage, and Cumulative percentage (Table 4), on the basis of which the distance biplot, scree plot, and Pearson correlation matrix were calculated. The biplot was prepared and is presented in (Figure 2).The perusal of the data by PCA reveals that the first two principlecomponents (PC) showed 92.3% cumulative variability, with the contribution of each principal component from PC1 to PC2 showing variability of 84.68% and 7.66%, respectively. The power of discrimination of the first PC was indicated by a high eigenvalue of 22.08 for axis 1 and 1.99 for the second PC. In first morphological analysis PC1 mainly represented by the root length, plant height, siliquae per plant, dry weight of leaves and leaf area all with loading coefficient greater than 0.25. PC2 represented by Number of seeds per siliques and number of leaves per plant with loading coefficient greater than 0.5. In PC1 all morphological parameters are positively correlated except siliquae length but PC2 was founded positively correlated with leaf area, number of leaves per plant, siliquae per plant, number of seeds per siliquae, dry weight of leaves and oil content.

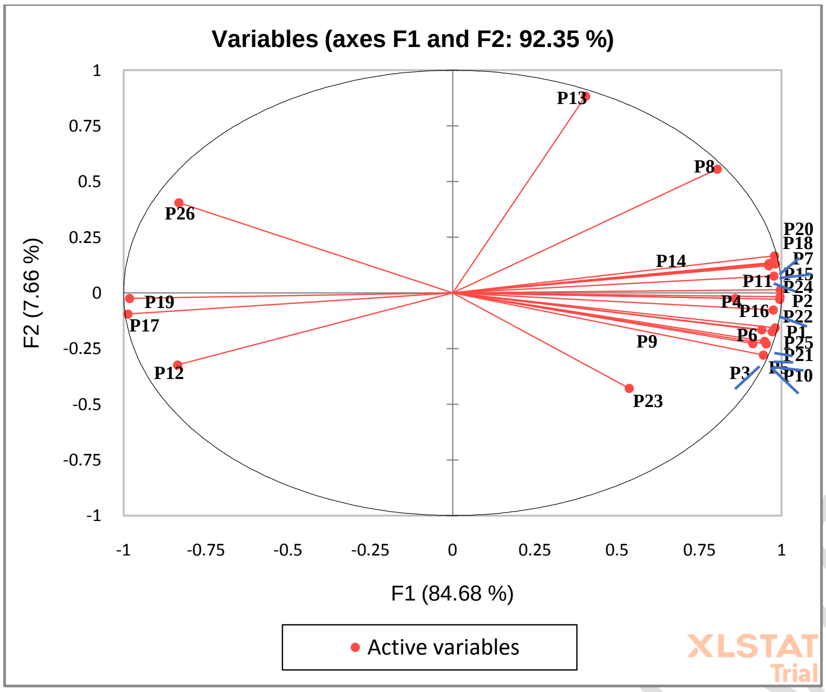

Figure2. Principal components of morphological, physiological and growth traits under well-watered (control) and drought stress and Early and late irrigated conditions.

(**P1=** Plant height**, P2=** Root length**, P3=** Fresh weight of leaves**, P4=** Dry weight of leaves**, P5=** Fresh weight of root**, P6=** Dry weight of root**, P7=** Leaf area**, P8=** Number of leaves per plant**, P9=** Number of primary branches**, P10=** Number of secondary branches, **P11**= Siliquae per plant, **P12**=Siliquae length, **P13**=Number of seeds per siliques, **P14**=1000 seed weight, **P15**=Oil content, **P16**=Relative water content (RWC), **P17**=Leaf Osmotic potential, **P18**=Membrane stability index (MSI), **P19**=Electrolyte leakage, **P20**=Leaf area index (LAI), **P21=**Crop growth rate (CGR), **P22=**Relative growth rate (RGR), **P23=**Net assimilatory rate (NAR), **P24=**Seed yield , **P25=**Biological yield , **P26=**Harvest index)

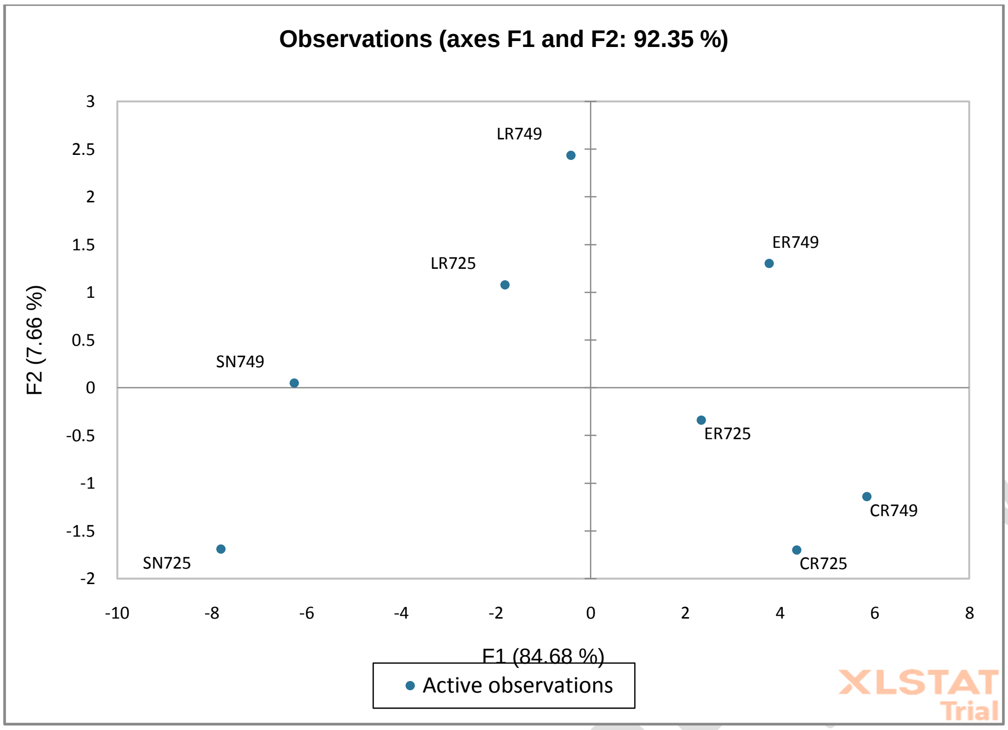

Figure3. Principal component analysis of morphological, physiological and growth traits under well-watered (control) and drought stress and Early and late irrigated conditions. The all parameters allow separating 2 cultivars that were either grown under well-watered (*circled*) or drought treatment (*box*) conditions. Early irrigated cultivars mainly RH-725 perform similar to control cultivars.

In second analysis (*i.e.* Relative water content, leaf osmotic potential, membrane stability index and electrolyte leakage) four different physiological traits studied in two different cultivars. PC1mainly represented by relative water content, electrolyte leakage and leaf osmotic potential all with loading coefficient greater than 0.80. PC2 explained by membrane stability index with loading coefficient greater 0.10. in third analysis (*i.e.*leaf area index, crop growth rate, relative growth rate, net assimilatory rate, seed yield, biological yield and harvest index). PC1 mainly represented by relative growth rate, harvest index, leaf area index and crop growth rate with loading coefficient greater than 0.96. PC2 represented by net assimilatory rate with factor loading 0.756. The bioplot of all 26 traits also revealed that as control and early irrigation regime perform morphologically almost similar, RH-725 cultivar behave morphologically and physiologically more similar to control irrigated cultivars. Morphological parameters contributed more than physiological parameters to the separation of control and other irrigation treatments. Among the 15 morphological parameters, plant height, number of secondary branches, dry and fresh weight of root and siliquae length were positively associated with the control group in both cultivars. In early irrigation treatment leaf area, number of leaves per plant, Siliquae per plant, 1000 seed weight and oil content were found to be positively correlated in cultivars. No morphological parameters contributed to the late irrigation treatment. Siliquae length was found to be positively correlated with drought treatment. Among the four physiological traits, relative water content, leaf osmotic potential, and membrane stability index were found to be positively correlated with the late irrigation regime in both cultivars. Membrane stability index was positively correlated with early irrigation regimes in both cultivars. Seven various growth yield parameters were also used for PCA, out of which only five parameters, *i.e.,*leaf area index, crop growth rate, relative growth rate, net assimilatory rate, and biological yield were found to be positively correlated in the control and early irrigation regimes.

On the basis of all studied parameters, early irrigation treatment in both cultivars was similar to control treatment. Among cultivars, RH-749 performs better in early and late irrigation treatments morphologically, physiologically, and in growth as compared to RH-725. Pearson's correlation matrix for 26 parameters was used for analysis and is represented in (Table S1) and (Figure 3). Almost all of the parameters were positively and significantly correlated with each other. But Morphological parameters (siliquae length) and physiological parameters like leaf Osmotic potential, electrolyte leakage, and growth parameter harvest index were found to significantly negatively correlate with almost all other parameters.

Table 4. Principal components showing the Eigen values, variability and cumulative variance

	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
Eigenvalue	22.018	1.992	1.012	0.644	0.223	0.081	0.031
Variability (%)	84.684	7.662	3.892	2.477	0.856	0.313	0.118
Cumulative %	84.684	92.345	96.237	98.714	99.570	99.882	100.000

Table5. Vector loadings and percent variation by principal components for morphological, physiological and growthparameters of *B. juncea.*

Characteristics / Code	**Principal components**				
	F1	**F2**	**F3**	**F4**	**F5**
Plant height (cm)/ (P1)	0.995	-0.017	-0.072	-0.028	0.012
Root length (cm) / (P2)	0.996	0.014	0.087	0.025	-0.003
Fresh weight of leaves (g) / (P3)	0.912	-0.228	-0.053	-0.319	0.005
Dry weight of leaves (g) / (P4)	0.975	-0.076	0.067	-0.168	0.101
fresh weight of root (g) / (P5)	0.948	-0.217	-0.178	0.132	0.011
dry weight of root (g) / (P6)	0.939	-0.165	-0.024	-0.264	0.124
Leaf area / (P7)	0.961	0.122	0.180	-0.131	-0.102
Number of leaves per plant / (P8)	0.804	0.556	-0.123	0.047	0.009
Number of primary branches / (P9)	0.945	-0.278	-0.153	-0.067	0.008
Number of secondary branches / (P10)	0.981	-0.157	-0.059	-0.077	0.025
Siliquae per plant / (P11)	0.981	0.131	-0.063	0.112	0.062
Siliquae length/ (P12)	-0.837	-0.323	-0.405	-0.172	-0.007
Number of seeds per siliquae/ (P13)	0.404	0.884	0.193	-0.120	-0.021
1000 seed weight / (P14)	0.978	0.166	-0.053	0.048	-0.024
Oil content / (P15)	0.972	0.135	0.164	0.086	-0.031
Relative water content (RWC) / (P16)	0.858	-0.024	-0.196	0.456	0.107
Leaf Osmotic potential / (P17)	-0.987	-0.095	0.097	-0.049	0.033
Membrane stability index (MSI) / (P18)	0.972	0.137	-0.183	-0.018	0.039

Electrolyte leakage/ (P19)	-0.983	-0.025	0.110	-0.051	-0.136
Leaf area index (LAI) / (P20)	0.962	0.132	0.144	-0.150	-0.107
Crop growth rate (CGR) / (P21)	0.953	-0.229	0.061	-0.188	0.007
Relative growth rate (RGR) / (P22)	0.994	-0.029	-0.034	-0.013	-0.098
Net assimilatory rate (NAR) / (P23)	0.536	-0.429	0.695	0.137	0.161
Seed yield / (P24)	0.976	0.076	-0.161	0.034	0.028
Biological yield / (P25)	0.972	-0.173	-0.098	0.089	-0.086
Harvest index / (P26)	-0.833	0.406	-0.111	-0.174	0.303

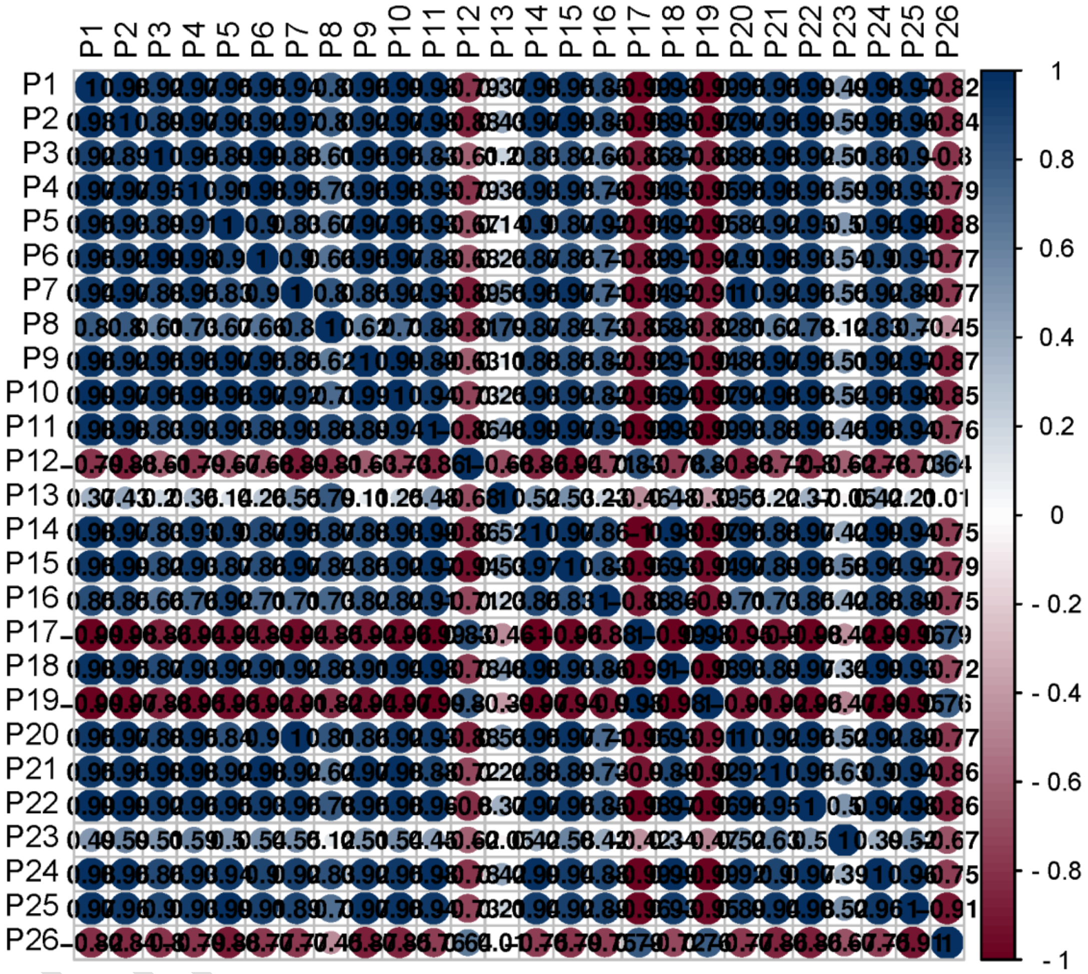

Figure4. Correlation matrix showed almost all characters positively significantly correlated with each other except silique length (P12), leaf osmotic potential (P17), electrolyte leakage (P19), and harvest index (P26).

(**P1=** Plant height**, P2=** Root length**, P3=** Fresh weight of leaves**, P4=** Dry weight of leaves**, P5=** Fresh weight of root**, P6=** Dry weight of root**, P7=** Leaf area**, P8=** Number of leaves per plant**, P9=** Number of primary branches**, P10=** Number of secondary branches,**P11**= Siliquae per plant, **P12**=Siliquae length, **P13**=Number of seeds per siliques, **P14**=1000 seed weight, **P15**=Oil content, **P16**=Relative water content (RWC), **P17**=Leaf Osmotic potential, **P18**=Membrane stability index (MSI), **P19**=Electrolyte leakage, **P20**=Leaf area index (LAI), **P21=**Crop growth rate (CGR), **P22=**Relative growth rate (RGR), **P23=**Net assimilatory rate (NAR), **P24=**Seed yield , **P25=**Biological yield , **P26=** Harvest index)

3.2 Heat map analysis

To identify the key parameters for assessing drought tolerance in *B. Juncea*, both morphological, physiological, and growth parameter measurements were used to plot a heatmap. As shown in (Figure 4), the morphological, physiological, and growth parameters of the 2 cultivars of *B. juncea*, grown under either early and late drought treatments or well-watered conditions (control), were used for hierarchical (row) clustering. When control cultivars were grown under well-watered conditions, the two cultivars clustered into group A, while the same set of two cultivars grown under drought conditions clustered into group B. This clear clustering demonstrates that, in comparison to control conditions, drought stress treatment alters both the morphology and physiological and growth characteristics of each cultivar. Interestingly, stressed cultivars were grouped in the same cluster, and early and late irrigated cultivars were grouped together in the same cluster (Figure 5).

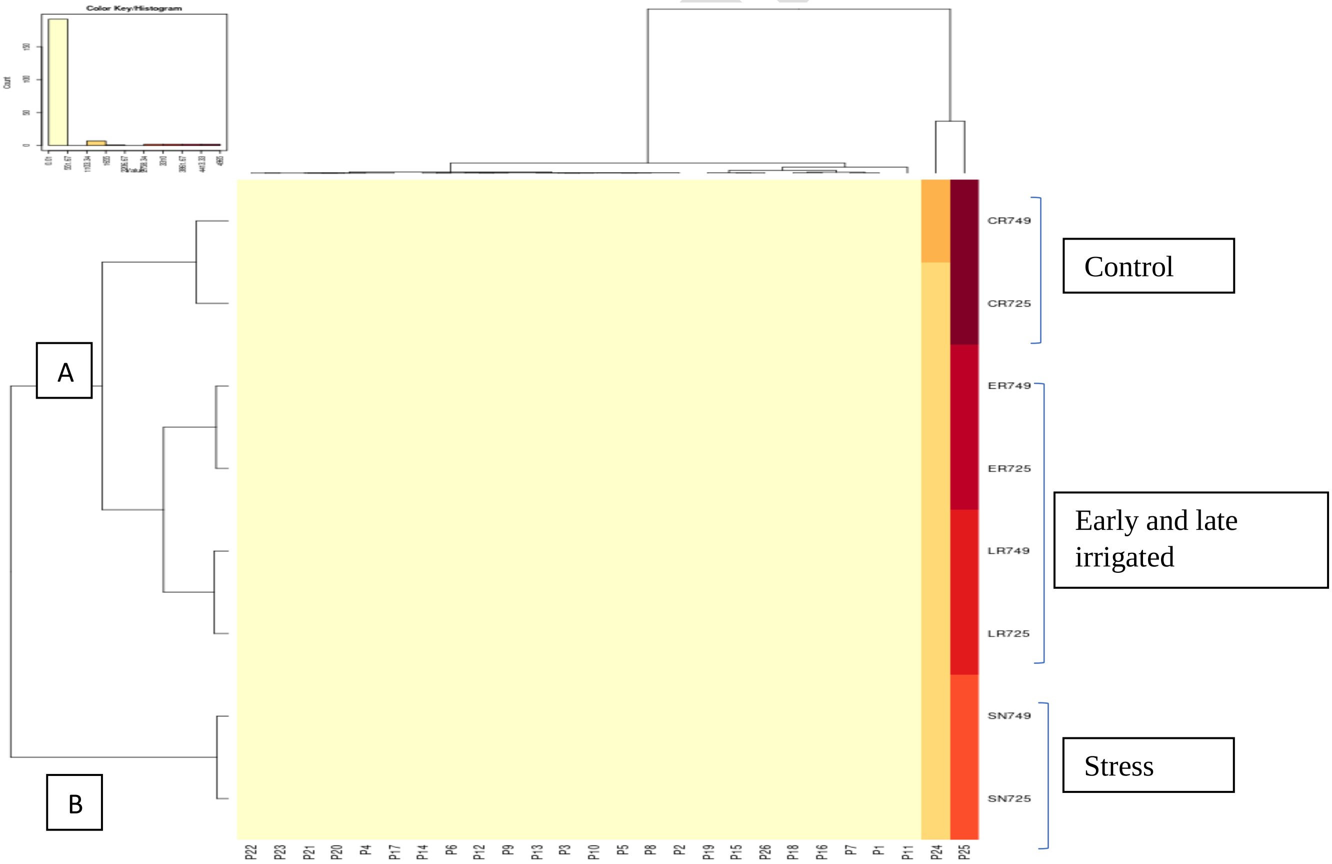

Figure 5. Hierarchical clustering and heat map for morphological, physiological and growth traits under well-watered (control) and drought stress both early and late conditions. Clustering analysis of variously treated cultivars (*left*) showed two main groups where the group A represents cultivars under the well-watered and irrigated single time condition; while group b represents those genotypes under

the stress treatment. The clustering analysis of various traits (*top*) showed three groups: group I and II includes morphological, physiological and growth parameters, group III include only two key growth traits seed and biological yield associate with drought tolerance.

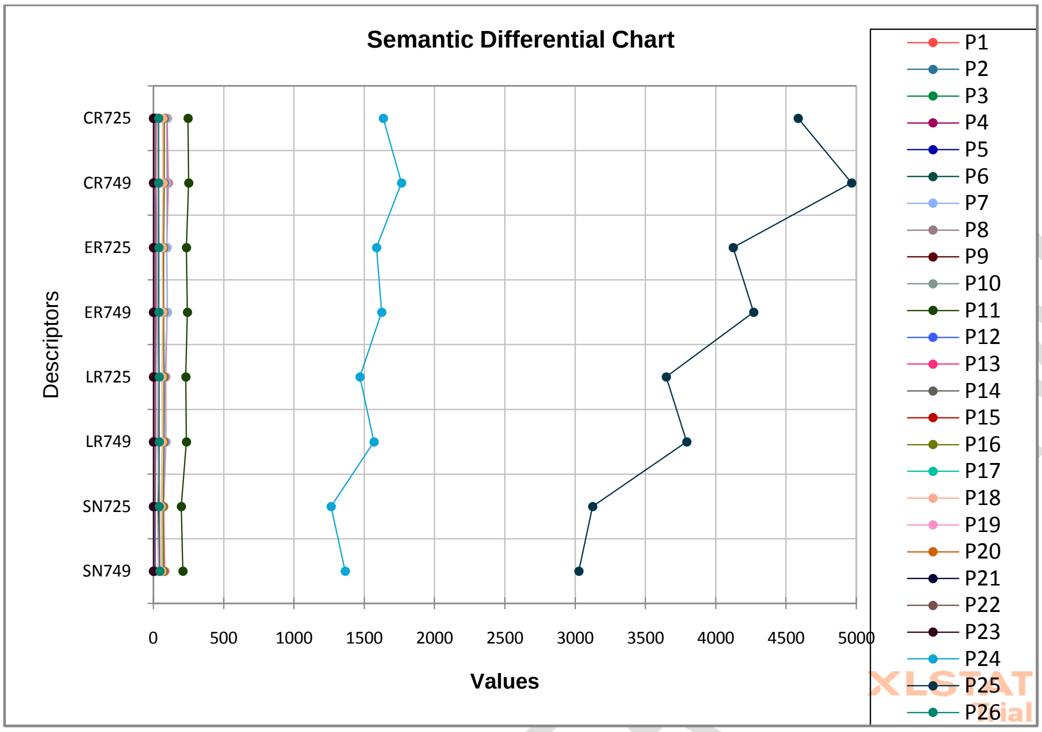

Figure6. Semanticdifferential chart showed the contribution of different traits in control, stress, early and late irrigated water regime in both cultivars. Most of the traits not so much effected in different stress condition except 924 and p25 traits.

(**P1=** Plant height**, P2=** Root length**, P3=** Fresh weight of leaves**, P4=** Dry weight of leaves**, P5=** Fresh weight of root**, P6=** Dry weight of root**, P7=** Leaf area**, P8=** Number of leaves per plant**, P9=** Number of primary branches**, P10=** Number of secondary branches, **P11**= Siliquae per plant, **P12**=Siliquae length, **P13**=Number of seeds per siliques, **P14**=1000 seed weight, **P15**=Oil content, **P16**=Relative water content (RWC), **P17**=Leaf Osmotic potential, **P18**=Membrane stability index (MSI), **P19**=Electrolyte leakage, **P20**=Leaf area index (LAI), **P21=**Crop growth rate (CGR), **P22=**Relative growth rate (RGR), **P23=**Net assimilatory rate (NAR), **P24=**Seed yield , **P25=**Biological yield , **P26=** Harvest index)

SN749= Stress treated RH-749 cultivar; SN725= Stress treated RH-725 cultivar; CR749= Control RH-749 cultivar; CR725= Control RH-725cultivar; ER749= Early irrigated RH-749 cultivar; ER749= Early irrigated RH-725cultivar; LR749=Late irrigated RH-749 cultivar; LR725=Late irrigated RH-725 cultivar

3.3 Agglomerative clustering

On the basis of the Euclidean distance matrix, utilising Ward's linkage method. The dendrogram was prepared and is presented in (Figure 6). Three clusters classified the cultivars on the basis of treatments; in the dendrogram, the distribution pattern was observed. Control cultivars were present in cluster I; Cluster II consisted of both early and late irrigated treatments; and stress-treated cultivars were found in cluster III. The highest cluster mean value was observed in cluster I for characters viz. plant height, leaf length, leaf width, flower diameter, petal length, and petal width. In cluster II, the greater mean value was for p8 and p13. Cluster III consisted of the accessions with the maximum mean for p12, 17, 19, and 26 (Table S2). Thus, the lowest-performing accessions with respect to number of characters were observed in cluster II.

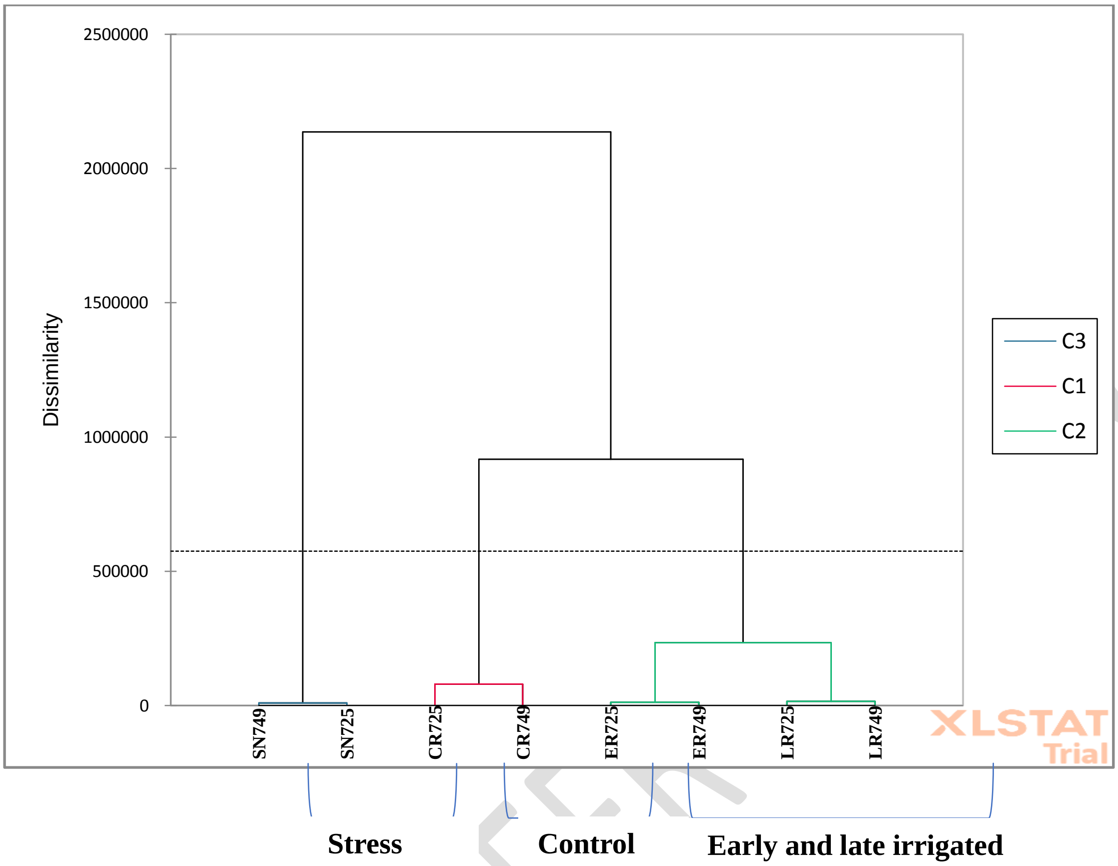

Figure 7. Agglomerative clustering dendrogram for morphological, physiological and growth traits under well-watered (control), drought stress and early and late conditions.

SN749= Stress treated RH-749 cultivar; **SN725**= Stress treated RH-725 cultivar;**CR749**= Control RH-749 cultivar;**CR725**= Control RH-725cultivar;**ER749=** Early irrigated RH-749 cultivar; **ER749=** Early irrigated RH-725cultivar; **LR749**=Late irrigated RH-749 cultivar; **LR725**=Late irrigated RH-725 cultivar

4. Conclusion

This study revealed that the level of irrigation markedly influenced the physiological, biochemical, and morphological features of the mustard cultivars studied, although the impact or effect upon these parameters varied according to the genotype, irrigation regime, and traits. The different irrigation regimes have significantly influenced the morphological, physiological, and growth traits of the two cultivars of *Brassica juncea*. The results indicate that water availability plays a crucial role in determining the overall growth and development of the plants and also reflect the importance of water management in maximising crop productivity. Cultivar-specific responses were observed, suggesting that certain cultivars may be more tolerant of or adapted to specific irrigation conditions. Water stress has deleterious consequences for the growth and development of both cultivars of *B. juncea* (L.) (RH-725 and RH-749). Out of both cultivars, the RH-749 cultivar performs very well with all irrigation regimes. Overall, the study highlights the importance of appropriate irrigation practises in *B.*

*juncea*cultivation. Optimised irrigation strategies can positively influence morphological and physiological traits, ultimately leading to improved growth and productivity in the two cultivars studied. The findings may contribute to better agricultural practises, especially in regions where water resources are limited or unpredictable. However, further research and validation under field conditions are necessary to generalise these results and implement them effectively in real-world scenarios.

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