Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10(2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

PHYSIOLOGICAL RESPONSES OF CANOLA (*Brassica napus* L.) GENOTYPES TO WATER DEFICIT

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ABSTRACT

Pakistan is primarily an agricultural country, with its economy heavily dependent on agricultural outputs. However, in the case of oilseed crops, the country spends a significant amount of foreign exchange on the import of edible oil. This situation highlights the urgent need to enhance domestic oilseed production through focused research efforts. To address this issue, research was conducted in the the Department

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Volume 3, Issue 10(2025)

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of Crop Physiology, University of Agriculture, Faisalabad, Pakistan. The main objective of this research was to evaluate the genotypic response of canola (Brassica napus L.) to moisture stress, which could be valuable in breeding programs aimed at developing drought-tolerant accessions. In this study, four canola accessions were evaluated under varying levels of moisture stress. The experiments were laid out in a completely randomized design with three replications. Three moisture stress treatments were applied: T_1 (100% field capacity), T_2 (75% field capacity), and T_3 (50% field capacity). For each accession in every replication, three plants were uprooted to record data. Physiological parameters including water potential, osmotic potential, turgor pressure, relative water content percentage, photosynthetic rate, transpiration rate, stomatal conductance, and sub-stomatal carbon dioxide (CO_2) conductance were measured. The analysis of variance revealed significant differences among the accessions for all traits except water potential, osmotic potential, and turgor pressure. The treatments showed significant effects on all measured traits, while the interaction between accessions and treatments was non-significant for most traits, indicating the presence of genetic variability in the breeding material.

Water potential values among accessions ranged from -17,416 to -19,575 MPa across all treatments. Under T_1, values ranged from -11,810 to -19,333 MPa; under T_2, from -1,176 to -24,569 MPa; and under T_3, from -14,822 to -28,678 MPa. Osmotic potential values ranged from -697 to -740 MPa across treatments, with T_1 ranging from -468 to -631 MPa, T_2 from -601 to -743.67 MPa, and T_3 from -846 to -1,075 MPa. Turgor pressure ranged from 16,701 to 18,835 MPa overall, with treatment-specific ranges of 11,342 to 18,702 MPa in T_1 and 11,160 to 23,820 MPa in T_2. Relative water content among accessions ranged from 37.89% to 53.07%. The photosynthetic rate ranged from 16.31 to 25.85 µmol/m²/sec. The transpiration rate varied from 2.13 to 4.61 µmol/m²/sec in T_1, 1.81 to 3.45 µmol/m²/sec in T_2, and 0.85 to 3.06 µmol/m²/sec in T_3. Stomatal conductance ranged from 0.05 to 0.22 µmol/m²/sec across treatments, with T_1 ranging from 0.06 to 0.16 µmol/m²/sec, T_2 from 0.07 to 0.22 µmol/m²/sec, and T_3 from 0.02 to 0.19 µmol/m²/sec. Sub-stomatal CO_2 conductance ranged from 136.92 to 332.5 µmol/m²/sec across treatments, with T_1 ranging from 157.44 to 368 µmol/m²/sec, T_2 from 133.78 to 343.11 µmol/m²/sec, and T_3 from 119.55 to 286.44 µmol/m²/sec.

The study concluded that the values of all physiological traits declined with increasing moisture stress. Among the tested accessions, Dunkeld and Zn-R-7 exhibited superior performance in terms of water potential, osmotic potential, photosynthetic rate, transpiration rate, stomatal conductance, and sub-stomatal CO_2 conductance. In contrast, Zn-N-6 and Zn-M-9 showed poor performance across these parameters. Based on these findings, it is suggested that the accessions Dunkeld and Zn-R-7 can be effectively utilized in breeding programs aimed at developing drought-tolerant canola varieties or hybrids.

Introduction

Edible oil is a vital household commodity and a cornerstone of national food security (Olabisi et al., 2021). As a major source of dietary energy and essential fatty acids, it plays a crucial role in human nutrition and overall health. Globally, the demand for vegetable oils continues to rise, driven by rapid population growth, urbanization, and

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evolving dietary habits (Mannucci et al., 2023). Among various oilseed crops, canola (Brassica napus L.) has emerged as a leading source of premium-quality edible oil due to its low saturated fat content and balanced composition of omega-3 and omega-6 fatty acids (Sachan et al., 2024).

In Pakistan, the consumption of edible oil has increased considerably over the past few decades. However, domestic production remains insufficient to meet national demand, with nearly 80–85% of the country's requirements being met through imports, primarily palm oil from Malaysia and Indonesia (Hussain et al., 2023; Goh & Potter, 2022). According to the Pakistan Bureau of Statistics (2023), the annual edible oil import bill exceeds USD 4.5 billion, contributing significantly to the national trade deficit (Mustafa & Iqbal, 2021). Despite possessing favorable agro-climatic conditions and extensive arable land, Pakistan's annual production of edible oil stands at only 0.5–0.7 million tons, mainly derived from cottonseed, sunflower, and canola. Among these, canola holds substantial potential due to its adaptability, high oil content (40–45%), and suitability for cultivation under both irrigated and rainfed conditions (Hameed & Azeem, 2017; Goyal et al., 2020).

Achieving self-sufficiency in edible oil production requires urgent attention to local oilseed development, particularly the expansion of canola cultivation (Sinha et al., 2020). However, one of the major challenges limiting canola productivity in Pakistan is moisture stress, especially in rainfed regions such as Potohar, Thal, and parts of southern Punjab and Balochistan (Rana et al., 2022). Erratic rainfall patterns and an increasing frequency of droughts due to climate change disrupt crop establishment, flowering, and seed development, ultimately leading to significant yield losses (Dietz et al., 2021).

Moisture stress is among the most critical abiotic stresses adversely affecting canola growth and productivity. It disrupts several physiological processes, including photosynthesis, nutrient uptake, water relations, and hormonal regulation (Sabagh et al., 2019). The impact of drought varies based on its severity, duration, and timing, as well as the genotype's inherent tolerance. Canola genotypes show differential responses to water-deficit conditions; while some are capable of maintaining higher relative water content, deeper root growth, and better osmotic regulation, others are more vulnerable (Mirzaei et al., 2013).

Understanding genotypic variation under moisture stress is essential for identifying drought-tolerant canola accessions suited to water-limited or rainfed environments in Pakistan (Nadeem et al., 2024). Genotypes that sustain physiological and agronomic performance under drought can serve as critical resources for breeding programs aimed at enhancing resilience (Cooper & Messina, 2023). Evaluating diverse accessions under controlled drought conditions can facilitate the screening of traits such as early vigor, root architecture, chlorophyll retention, water-use efficiency, and yield stability.

In Pakistan, most research on canola has focused on agronomic practices and hybrid development, with relatively limited emphasis on drought physiology and genotype screening. This creates a critical research gap, particularly in the identification and utilization of locally adapted, stress-resilient germplasm. Considering the country's escalating water scarcity and declining irrigation resources, expanding canola cultivation into marginal areas will depend on the development and promotion of

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drought-tolerant genotypes.

This study aims to evaluate the genotypic response of diverse canola accessions to moisture stress under local agro-climatic conditions. The specific objectives are to: (i) assess key physiological traits under both optimal and drought-stressed environments, (ii) quantify the degree of drought tolerance among the accessions, and (iii) identify promising genotypes for future breeding and commercial cultivation. By addressing this challenge within the national context, the study seeks to contribute directly to enhancing domestic oilseed production, reducing dependency on imports, and improving national food and economic security.

The findings from this research will offer critical insights into the adaptive mechanisms of canola under water stress and support the development of climate-resilient varieties. Promoting drought-tolerant canola genotypes will strengthen the sustainability of oilseed cropping systems in Pakistan's arid and semi-arid regions, aligning with broader goals of climate-smart agriculture and economic stability.

MATERIAL AND METHODS

This experiment was conducted in wire house in pots and laid out in triplicate completely randomized design in factorial arrangements. Five seeds of the two brassica accessions were sown in plastic pots having 2kg sand as a growth medium. First 15 days of germination normal irrigation was applied. After 15 days of sowing three treatments of water stress were applied T_1 (100% FC), T2 (75% FC) and T3 (50% FC). Three plants were uprooted after 45 days of sowing for data recording.

Water Potential (MPa)

Leaf water potential was measured with water potential apparatus (Chas W. Cook & Sons. Birmingham B 42, ITT England) available in the Department of Botany, University of Agriculture, Faisalabad, A single leaf (flag leaf) was sealed in the pressure chamber with the cut surface protruding out of the hole. Pressure was applied to the leaf from a cylinder of compressed gas until xylem sap appeared at the cut surface. This balancing pressure was regarded as the tension originally existing in the xylem sap and approximately equal to water potential of the cells. Sampling was done between 6.00 and 9.00 a.m. to avoid evaporation losses. The leaves were placed in the pressure chamber as quickly as possible and measurements were made on flag leaf from control and stressed treatments separately.

Osmotic potential (MPa)

The same leaf as used for water potential, was frozen in a freezer below -20 °C for more than seven days, after which the frozen leaf materials was thawed and cell sap was extracted by pressing the material with the help of glass rod. A drop of sap was used directly for the determination of osmotic potential in a vapor pressure osmometer (Osmette S-Japan) calibrated in mOsmol kg.

Turgor pressure (MPa)

The turgor potential was calculated by using the formula given by Hopkin (1999) as the difference between water potential and osmotic potential values.

Turgor potential = Water Potential - Osmotic potential =

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$\Psi p = \Psi w - \Psi s$

Relative water content (RWC %)

Each sample containing a flag leaf was taken from each pot. Fresh weight of each sample was taken. Leaf was dipped in test tube containing water for 15-18 hours. Then it was wiped with the tissue paper and turgid weight was taken. The samples were dried for 3 days at 72 °C then dry weight of each sample was taken. For each pot relative water content was calculated by using the formula given by Karrou and Maranville, 1995.

RWC = (Fresh weight- Dry weight/ Turgid weight-Dry weight) × 100

Gas exchange parameters

Photosynthetic rate (A), Transpiration rate (E), Stomatal conductance and sub-stomatal CO_2 conductance were recorded by using portable infrared gas analyzer (IRGA) model ADC Bio- Scientific LCi-SD System Serial No.33585.

Statistical Analysis

The recorded data were subjected to Analysis of the variance. The data were subjected to following analyses of variance (Steel et al., 1997) to estimate the variations among the genotypes. The significant data were further subjected to Duncan's New Multiple Range Test.

Genotypic variance (VG) = AMS-EMS/r
Phenotypic variance (VP) = VE+VG
Environmental variance (VE) = EMS
Where
AMS = Accession mean square
EMS = Error mean square

RESULTS AND DISCUSSION

Water Potential (MPa)

The Table 4.8(a) presents the analysis of variance of canola accessions for water potential across different levels of moisture stress. Mean comparison of accessions for water potential is resented in Fig 4.10 and Appendix 6. The analysis of variance showed that there were non-significant differences among the accessions across the treatments. But the treatments and their interaction with accessions showed significant differences. Kumar and Jelston (1992), Good and Maclean (1993), Kumar et al. (1994) and Chartzoulakis et al. (1999) found the significant ferences among the accessions. The water potential of the accessions ranged from -17416 to19575 MPa across the treatments

Mean comparisons indicated that the water potential was decreased by increasing the moisture stress levels. The T_1 (100% FC), T_2 (75% FC) and T_3 (50% FC showed -15600 MPa, 85 MPa and -21911 MPa water potential respectively and significantly different from each other except T1 (100% FC) and T2 (75% FC).

Mean squares values from the analysis of variance for water potential under different moisture stress levels are shown in Table 4.8(b). Accessions showed significant differences in all the treatments except T_1 (100% FC). In T_1 (100% FC) the water

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potential ranged from-11810 to-19333 MPa. In T2 (75% FC) the water potential ranged from -11761 to -2456 MPa. The accession Dunkeld showed the maximum water relation which was significantly different from Zn-M-9 and Zn-N-6. While Zn-N-6 showed minimum water potential in T2 (75% FC) and significantly different from the other accessions except Zn-M-9. In T3 (50% FC), the water potential ranged from 14822 to 28678 MPa. The accession Dunkeld showed the maximum water potential in T3 (50% FC) and significantly different form Zn-M-9 and Zn-N-6. While Zn-N-6 showed minimum water potential followed by Zn-M-9 and significantly different from other accessions in T3 (50% FC). The results showed that water potential decreased by increasing moisture stress. Maximum decrease in water relation at 50% FC level. Nourouzi et al (2008) and Singh et al (2009) found that the effect of stress on plant is usually perceived as decrease in water potential. So our findings are according to the literature.

Table 4.8(a) Analysis of variance of canola accessions for water potential across different moisture stress levels

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatments (T)	2	4.426	1.213	11.98**
Accessions (A)	3	2.702	9006559	0.89
T × A	6	1.063	1.773	
Error	24	2.430	1.012	17.52**
Total	35	1.577		

** = Significant at 1% probability level

Table 4.8(b) Mean squares values from analysis of variance of canola accessions for water potential under different moisture levels

Sources of variation	Degrees of freedom	Treatments		
		T_1 (100% FC)	T2 (75% FC)	T3 (50% FC)
Accessions	3	4.33	1.41**	1.79**
Error	8	2.59	2156035	2277710

* = Significant at 5% probability level

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Online ISSN 3007-3197
Print ISSN 3007-3189

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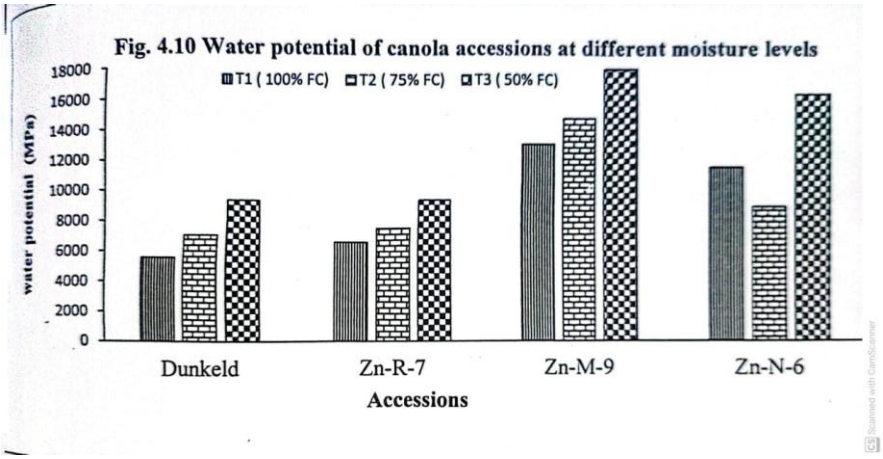

Osmotic Potential (MPa)

The Table 4.9(a) presents the analysis of variance of canola accessions for osmotic potential across different levels of moisture stress. Mean comparisons of accessions for osmotic potential are presented in Fig 4.11 and Appendix 7. The analysis of variance showed that there non-significant differences in osmotic potential of the accessions across the treatments. But treatments and their interaction with accessions showed significant differences. Siddiqui et al (2008), Nourouzi et al. (2008) were found significant variations among accessions. The osmotic potential of the accessions was recorded from -6979 to-7403 MPa. Mean comparisons indicated that the osmotic potential was decreased by increasing the water stress. The treatments T1 (100% FC), T_2 (75% FC) and T3 (50% FC) showed -546.25-663.83 and -942.69MPa respectively and significantly different from each other.

Mean square values from the analysis of variance for osmotic potential under different moisture stress levels are shown in Table 4.9(b). The accessions showed significant differences all the treatments except T_2 (75% FC), In T1 (100% FC) the osmotic potential ranged from -468 to -631 MPa. The accession Dunkeld showed maximum osmotic potential in T1 (100% FC) and significantly different from the accession Zn-N-6. While the accession Zn-N-6 showed minimum osmotic potential and significantly different from the accession Dunkeld in T1 (100% FC). In T_2 (75% FC) the osmotic potential ranged from -601 to-743.67 MPa. All the accessions showed non- significant in T_2 (75% FC). In T3 (50% FC) the osmotic potential ranged from -846 -1075 MPa. The accession Dunkeld showed the maximum osmotic potential which was significantly different form Zn-N-6. Whereas, the accession Zn-N-6 showed minimum osmotic potential and significantly different from the accession Dunkeld.

The results showed that osmotic potential decreased by increasing moisture stress Maximum decrease in osmotic potential at 50% FC level. Phutella et al. (2000). Kouser et al. (2006), Siddiqui et al (2008) Nourouzi et al. (2008), investigated that there was a decrease in leaf osmotic potential with the onset of the drought stress and osmotic potential was lower by increasing the drought stress. So our findings are according to the literature.

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Table 4.9(a) Analysis of variance of canola accessions for osmotic potential across different moisture stress levels

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatments (T)	2	995027	497514	130.08**
Accessions (A)	3	8188	2729	0.71
A × T	6	164404	27401	7.16**
Error	24	91795	3825	
Total	35	1259415		

** = Significant at 1% probability level

Table 4.9(b) Mean squares values from analysis of variance of canola accessions for osmotic potential under different moisture levels

Sources of variation	Degrees of freedom	Treatments		
		T_1 (100% FC)	T2 (75% FC)	T3 (50% FC)
Accessions	3	15008	12643	29878*
Error	8	1920	3447	6107

* = Significant at 5% probability level

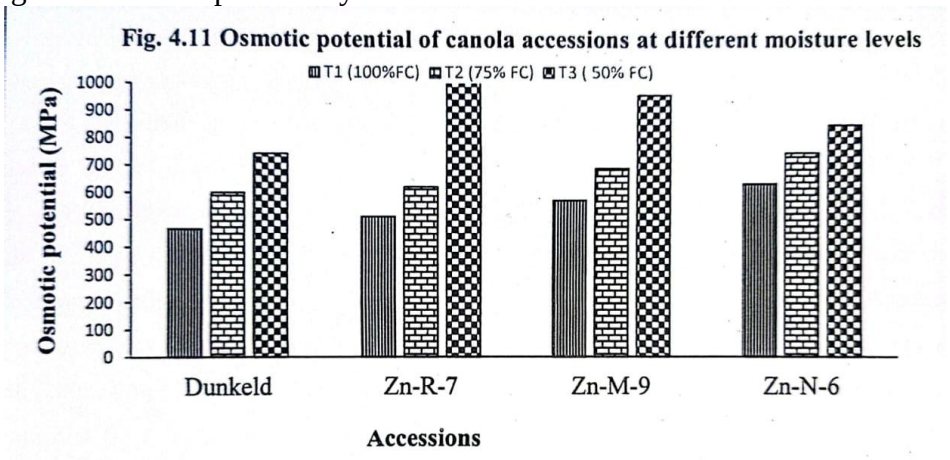

Fig. 4.11 Osmotic potential of canola accessions at different moisture levels

Turgor Pressure (MPa)

The Table 4.10(a) presents the analysis of variance of canola accessions for turgor pressure across different levels of moisture stress. Mean comparisons of accessions for osmotic potential are presented in Fig 4.12 and Appendix 8. The analysis of variance showed that there were non-significant differences in osmotic potential of the accessions across the treatments. But the treatments and their interaction with accessions showed highly significant differences. The osmotic potential of the accessions was recorded from 16701 to 18835MPa Accessions showed non-significant differences. The treatments T1 (100% FC), T2 (75% FC) and T3 (50% FC) showed 15053, 17421 and 20968 MPa respectively and significantly different from each other except the difference between T1 (100% FC) and T2 (75%FC).

Mean square values from the analysis of variance for osmotic potential under different posture stress levels are shown in Table 4.10(b). The accessions showed significant

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differences all the treatments except T1 (100% FC). In T1 (100% FC) the turgor pressure ranged from 11342 15053 MPa. In T2 (75% FC) the turgor pressure ranged from 11160 to 23826 MPa. In T_2 (75% FC) the turgor pressure ranged from 13976 10 27602 MPa. The Zn-N-6 showed maximum turgor pressure in T_2 (75% FC) and significantly different from Dunkled and Zn-R-7. Whereas, the accession Dunkeld showed minimum turgor pressure and significantly different from the accessions Zn-M-9 and Zn-N-6 in T_2 (75% FC), In T_1 (50% FC) treatment the accession Dunkled showed maximum turgor pressure and significantly different from Zn-M-9 and Zn-N-6. While Zn-N-6 had minimum turgor pressure and significantly different from the accessions Dunkled Zn-R-7 in T3 (50% FC).

Maximum increase in turgor pressure at 50% FC level. Kumar and Jelston (1992), Good and Maclagan (1993) investigated that there was a decrease in leaf osmotic potential with the onset of the drought stress. Phutella et al. (2000). Kouser et al. (2006). Siddiqui et al (2008) stated that osmotic potential was lower by increasing the drought. Nourouzi et al. (2008), Siddiqui et al. (2008) found that the effect of stress on plant is usually perceived as a decrease in water potential. So our findings are according to the literature.

Table 4.10(a) Analysis of variance of canola accessions for turgor pressure across different moisture stress levels

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatments (T)	2	2.12	1.063	10.51**
Accessions (A)	3	2.63	8787009	0.87
A × T	6	1.04	1.73	17.14**
Error	24	2.48	1.01	
Total	35	1.52		

** = Significant at 1% probability level

Table 4.10(b) Mean squares values from analysis of variance of canola accessions for turgor pressure under different moisture levels

Sources of variation	Degrees of freedom	Treatments		
		T_1 (100% FC)	T2 (75% FC)	T3 (50% FC)
Accessions	3	1.25	1.38	1.75**
Error	8	2.08	2018610	22544567

** = Significant at 1% probability level

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Print ISSN 3007-3189

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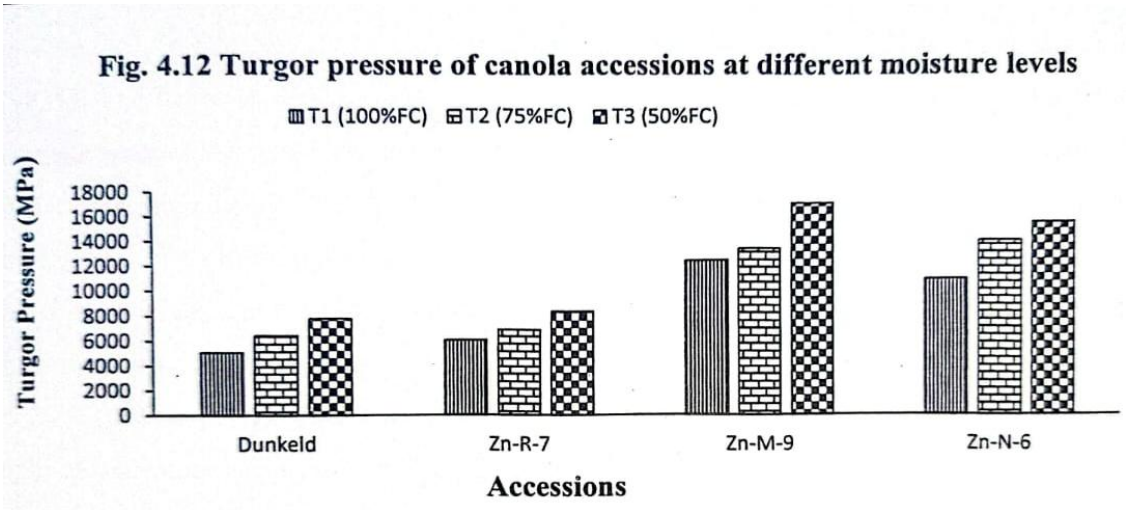

Relative water contents (RWC %)

The Table 4.11(a) presents the analysis of variance of canola accessions for relative water contents across different levels of moisture stress Mean comparisons of canola accessions for relative water contents are presented in Fig 4.13 and Appendix 9. The analysis of variance of canola for relative water contents indicated that there were significant differences relative water contents of the accessions and treatments. But the Interaction between accessions treatments were non-significant. Kumar and Jelston (1992), Ghaffari et al. (2007), Ullha et al. (2012) and Yu et al (2013) also found the significant differences among the accessions. The relative water contents of the accessions was recorded from 37.88 to 53.07%. The accession Dunkeld showed maximum relative water contents and significantly different from other accessions. While Zn-N-6 showed minimum relative water contents and significantly different from all other accessions

Mean comparison of accessions for relative water contents is presented in Fig 4.14. The treatments T_1 (100% FC), T_2 (75% FC) and T_3 (50% FC) showed relative water contents 50.8, 41.50 and 37.5% respectively and significantly different from each other.

Mean squares values from the analysis of variance for relative water contents under different moisture stress levels are presented in Table 4.11(b) Accessions showed significant differences in all the treatments. In T1 (100% FC) the relative water contents ranged from 42.67 62.23%. The accession Dunkeld showed maximum relative water content and significantly afferent from all the other accessions in T1 (100%FC). Minimum relative water contents were observed for Zn-M-9 and significantly different from all the other accessions except Zn-N-6. In T_2 (75% FC) the relative water contents ranged from 36.66 to 50.66%. Maximum relative water contents were observed for the accession Dunkeld and it was significantly different from all the other accessions in T2 (75% FC). The accession Zn-N-6 showed minimum relative water contents and significantly different from all the other accessions in T2 (50% FC) treatment. In T3 (50% FC) the relative water contents ranged from 34 to 46.33%. The accessions Dunkeld showed the maximum relative water contents which was significantly different all the other accessions in T3 (50% FC). Whereas, Zn-N-6 showed minimum relative water contents and significantly

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different from all the other accessions in T3 (50% FC). The results showed that relative water contents were decreased by increasing moisture stress. Maximum decrease in relative water contents in T3 (50% FC) level. Kumar and Jelston (1992), Kumar et al. (1994), Kumat et al. (1993) reported RWC% was decreased with increase in moisture stress in Brassica juncea. Ullha et al. (2012) and Yu et al (2013) reported that significant reduction in RWC% of Brassica napus L. by increasing the moisture stress, Ghaffari et al. (2007), Saensee et al. (2012) also investigated that relative water contents were decreased by increasing the drought stress in sun flower. So our findings are according to the literature.

Table 4.11(a) Analysis of variance of canola accessions for relative water contents across different moisture stress levels

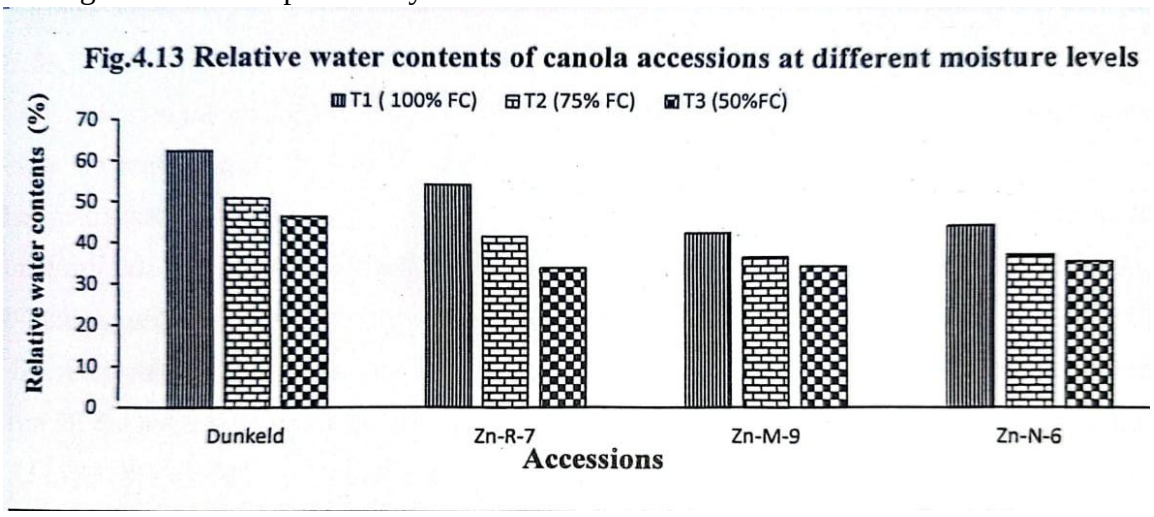
Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatments (T)	2	1117	558	33.80**
Accessions (A)	3	1306	435	26.34**
A × T	6	153	25.63	1.55
Error	24	396	16.51	
Total	35	2976		

** = Significant at 1% probability level

Table 4.11(b) Mean squares values from analysis of variance of canola accessions for relative water contents under different moisture levels

Sources of variation	Degrees of freedom	Treatments		
		T_1 (100% FC)	T2 (75% FC)	T3 (50% FC)
Accessions	3	253.99**	127.66*	105**
Error	8	11.59	23.50	14.50

** = Significant at 1% probability level
* = Significant at 5% probability level



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Photosynthetic rate (µmol/m^2/sec)

The Table 4.12(a) presents the analysis of variance of canola accessions for photosynthetic rate across different levels of moisture stress. Mean compressions of canola for photosynthetic rate in Fig 4.14 and Appendix 10. The analysis of variance indicated that there were significant differences in photosynthetic rate of the accessions and treatments. But the interactions across the treatments were non-significant. Xu et al. (1996), Kousar et al. (2006), Nourouzi et al. (2008), Siddiqui et al. (2008), Anjum et al. (2011), Ullha et al., 2012), Shabani et al. (2013) and Yu et al. (2013) also found the significant variation among the accessions. Photosynthetic rate of accessions ranged from 9.36 to 33.90 µmol/m²/sec in present breeding material. The accession Dunkeld had maximum photosynthetic rate and significantly different from all the other accessions. While the accession Zn-N-6 had minimum photosynthetic rate and significantly different from Dunkeld and Zn-R-7.

Mean comparisons indicated that photosynthetic rate was decreased by increasing the moisture stress levels. The) treatments T_1 (100% FC), T_2 (75% FC) and T_3 (50% FC showed 25.85. 20.51 and 16.31 (µmol/m²/sec) respectively and significantly different from each other.

Table 4.12(a) Analysis of variance of canola accessions for photosynthetic rate across different moisture stress levels

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatments (T)	2	549	274	29.86**
Accessions (A)	3	4337	1445	157**
A × T	6	113	18.85	2.05
Error	24	220	9.20	
Total	35	5220		

** = Significant at 1% probability level

Table 4.12(b) Mean squares values from analysis of variance of canola accessions for photosynthetic rate under different moisture levels

Sources of variation	Degrees of freedom	Treatments		
		T_1 (100% FC)	T2 (75% FC)	T3 (50% FC)
Accessions	3	611	547.41	324.93**
Error	8	12.07	9.22	6.29

** = Significant at 1% probability level

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Online ISSN 3007-3197
Print ISSN 3007-3189

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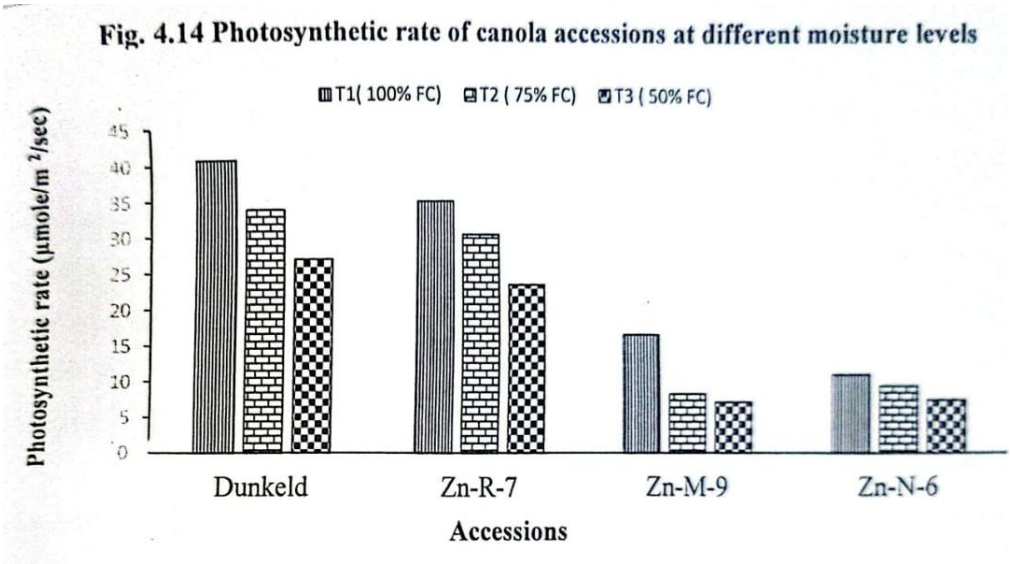

Fig. 4.14 Photosynthetic rate of canola accessions at different moisture levels

Mean squares values from the analysis for photosynthetic under different moisture stress levels are presented in Table 4.12(b). Accessions showed highly significant differences in all the treatments. In treatment T: (100% FC) photosynthetic rate ranged from 11.06 to 40.80 µmol/m^2/ sec. The accession Dunkeld showed maximum photosynthetic rate followed by Zn-R-7and significantly different from the accessions Zn-M-9 and Zn-N-6 in T. (100% FC). Minimum photosynthetic rate was observed for the accession Zn-N-6 and significantly different from all the other accessions except Zn-M-9 in T_1 (100% FC). Photosynthetic rate ranged from 8.33 to 33.90 µmol/m^2/sec in the treatment T2 (75% FC). The accession Dunkeld had maximum photosynthetic rate in T (75% FC) and significantly different from other accessions except Zn-R-7. Minimum photosynthetic rate was observed for Zn-M-9 followed by Zn-N-6 in T2 (75%) and significantly different from other accessions. In treatment T3 (50% FC) photosynthetic ate and significantly different from all other accessions in T3 (50% FC). The accession Zn-M. ranged from 7.20 to 27 µmol/m^2/sec. The accession Dunkeld showed maximum photosynthetic showed minimum photosynthetic rate followed by Zn-N-6 and significantly different from all the other accessions. Results showed that with increase in moisture stress photosynthetic rate was decreased, maximum reduction was observed at T_3 (50% FC). Maximum reduction of photosynthetic rate was observed in susceptible accessions ie. Zn-M-9 and Zn-N-6. Kumar and Jelston (1992), Good and Maclagan (1993), Kumar et al (1994), Chartzoulakis et al. (1999). Photela et al. (2000), Kousar et al. (2006), Nourouzi et al. (2008), Siddiqui et al. (2008), Anjum aal (2011), (Ullha et al., 2012), Shabani et al. (2013) and Yu et al. (2013) found significant differences in material for this trait. Xu et al. (1996) stated that decrease in applied water resulted lower the photosynthetic rate, our findings are according to the literature and suitable further breeding programs.

Transpiration rate (µmol/m^2/sec)

The Table 4.13(a) presents the analysis of variance of canola accessions for transpiration rate across different levels of moisture stress. Mean compressions of

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canola for transpiration ale presented in Fig 4.15 and Appendix 11. The analysis of variance of transpiration rate indicated that there were significant differences in transpiration rate of the accessions and treatments. But their interactions across the treatments were non-significant. Kumar et al. (1994), Chartzoulakis et al. (1999), Singh et al. (2009), Anjum et al. (2011). Qaderia et al., (2011), Ullha et al., 2012), Shabani et al. (2013) and Yu et al. (2013 found significant differences among accessions. Transpiration rate ranged from 1.67 to 3.71 µmol/m²/sec) in present breeding material. Dunkeld had maximum transpiration rate and significantly different from all the other accessions except Zn-R-7. Zn-N-6 had minimum transpiration rate and significantly different from Dunkeld and Zn-R-7.

Mean comparisons indicated that transpiration rate was decreased by increasing the moisture stress levels. The T. (100% FC), T2 (75% FC) and T3 (50% FC) treatments showed 334, 2.66 and 1.97 µmol/m²/sec respectively and significantly different from each other.

Table 4.13(a) Analysis of variance of canola accessions for transpiration rate across different moisture stress levels

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatments (T)	2	11.23	5.61	40.05**
Accessions (A)	3	25.26	8.42	60.06**
A × T	6	1.38	0.23	1.65
Error	24	3.36	0.14	
Total	35	41.26		

** = Significant at 1% probability level

Table 4.13(b) Mean squares values from analysis of variance of canola accessions for transpiration rate under different moisture levels

Sources of variation	Degrees of freedom	Treatments		
		T_1 (100% FC)	T2 (75% FC)	T3 (50% FC)
Accessions	3	3.41**	2.25**	3.22**
Error	8	0.16	0.15	0.10

** = Significant at 1% probability level

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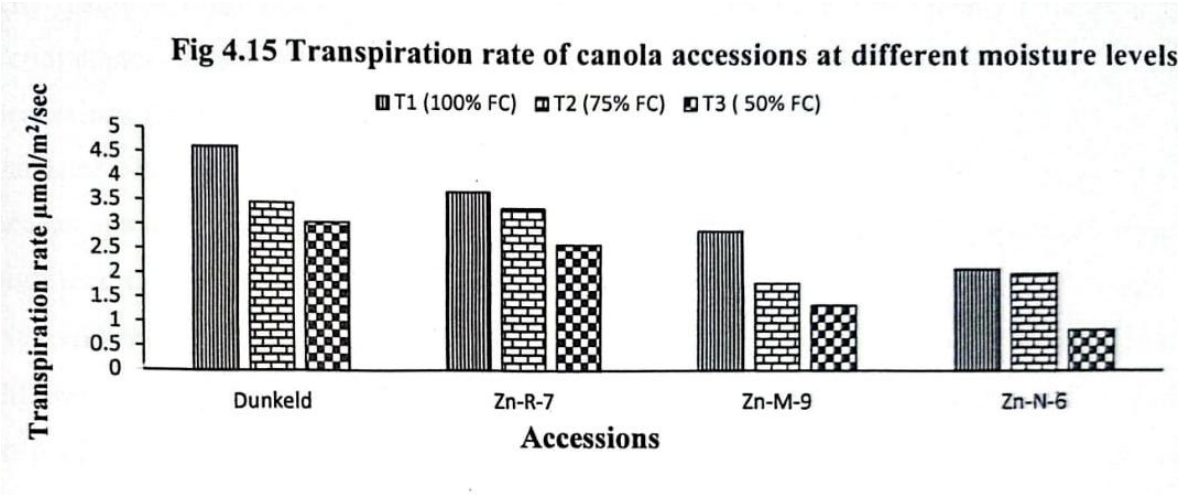

Mean squares values from the analysis for transpiration rate under different moisture stress levels are shown in Table 4.13(b). The accessions showed significant differences in all the treatments. In treatment T, transpiration rate ranged from 2.13 to 4.61 µmol/m^2/sec. The accession Dunkeld showed maximum transpiration rate and significantly different from other accessions in T: (100% FC). Minimum transpiration rate was observed for Zn-N-6 followed by Zn-M-9 and significantly different from all the other accessions in T1 (100%FC). The transpiration rate in T2 (75% FC) ranged from 1.81 to 3.45 µmol/m^2/sec. The accession Dunkeld showed maximum transpiration rate and significantly different from all other accessions in T_2 (75% FC). Minimum transpiration rate was observed for Zn-M-9 and significantly different from all the other accessions in T_2 (75%FC). In T_3 (50% FC) transpiration rate ranged from 0.85 to 3.06 µmol/m^2/sec. The accession Dunkeld showed maximum transpiration rate and significantly different from all other accessions in T_3 (50% FC). The accession Zn-N-6 showed minimum transpiration rate and significantly different from all the other accessions. Results showed that with increase in moisture stress transpiration rate was decreased, maximum reduction was observed at T_3 (50% FC) Kumar et al. (1994), Chartzoulakis et al. (1999), Singh et al. (2009), Anjum et al. (2011), Qaderia et al., (2011), Ullha et al., 2012), Shabani et al. (2013) and Yu et al. (2013) found that transpiration rate was decreased by increasing the moisture stress levels.

Stomatal conductance (µmol/m^2/sec)

The Table 4.14(a) presents the analysis of variance of canola accessions for stomatal conductance across different levels of moisture stress. Mean comparisons of canola accessions for stomatal conductance presented in Fig 4.16 and Appendix 12. The analysis of variance showed that there were significant differences in stomatal conductance of the accessions and treatments. But the interaction between accessions and treatment were non-significant. Uperty and Tomar (1993), Mogenson et al. (1996), Kousar et al. (2006). Naderikharaji et al. (2008). Anjum et al. (2011) and Riaz et al. (2013) reported the significant differences among the accessions. Stomatal conductance of the accessions ranged from 0.05 to 0.22 µmol/m²/sec. The accession Dunkeld showed maximum stomatal conductance and significantly different from all the other accessions. Minimum stomatal conductance was observed for the accession

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Zn-N-6 and significantly different from Dunkeld and Zn-R-7.

Table 4.14(a) Analysis of variance of canola accessions for stomatal conductance across different moisture stress levels

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatments (T)	2	24990	12495	30.16**
Accessions (A)	3	206832	68944	166.43**
A × T	6	2140	356	0.86
Error	24	9942	414	
Total	35	2439		

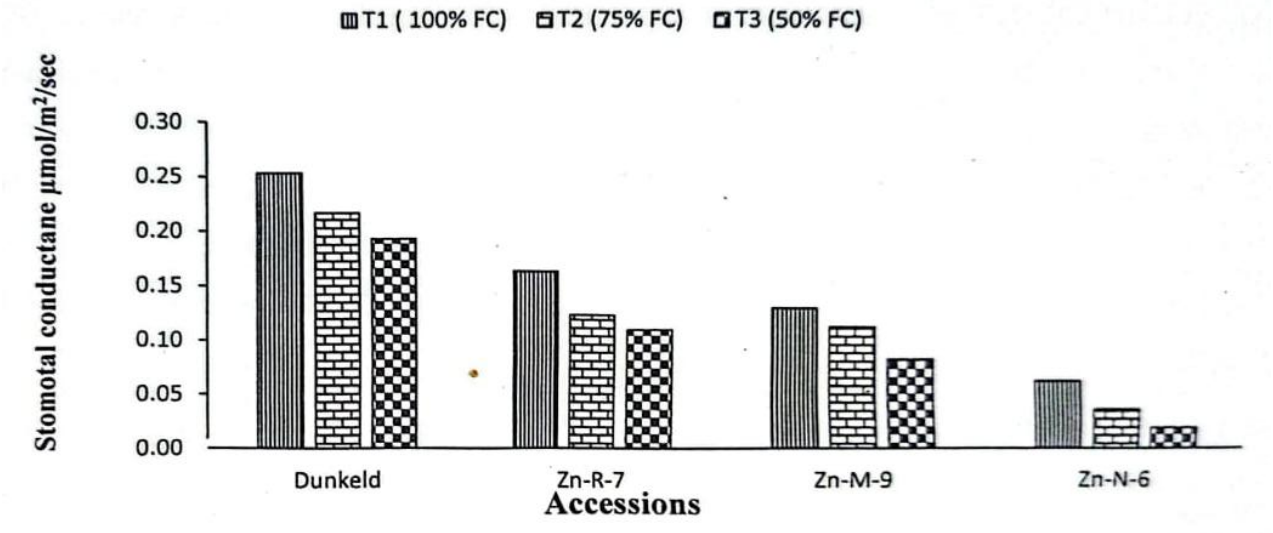
** = Significant at 1% probability level

Table 4.14(b) Mean squares values from analysis of variance of canola accessions for stomatal conductance under different moisture levels

Sources of variation	Degrees of freedom	Treatments		
		T_1 (100% FC)	T2 (75% FC)	T3 (50% FC)
Accessions	3	0.018	0.016	0.015**
Error	8	0.0002	0.0001	0.0009

** = Significant at 1% probability level

Fig. 4.16 Stomotal conductance of canola at different moisture levels



Mean comparison indicated that stomatal conductance was decreased trying the e stress levels. The treatments T1 (100% FC), T2 (75% FC) and T3 (50% FC) showed 0.15, 0.13 and 0.10 µmol/m^2/sec respectively and significantly different from each other except the difference b/w T2 (75% FC) and T3 (50% FC). Mean square values from the analysis of variance for stomatal conductance under different moisture levels

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are shown in Table 4.14(b). The accession Dunkeld showed the maximum stomatal conductance which was significant different from all the accession in T1 (100% FC). While Z-N-6 showed mum stomatal conductance and significantly differences from all the other accessions in T1 (100 FC). In T_2 (75% FC) the stomatal conductance ranged from 0.07 to 0.22mol/m²/sec. The accession Dunkeld showed the maximum stomatal conductance which was significantly of significantly different from all the other accessions. Whereas, Zn-N-6 showed minimum stomatal conductance. The stomatal conductance ranged from 0.02 to 0.19 µmol/m^2/ses in T_3 (50% FC). The accessions Dunkeld showed the maximum stomatal conductance which was significantly different from all the other sons while Zn-N-6 showed minimum stomatal conductance in T_3 (50% FC). The results showed that stomatal conductance decreased by increasing moisture stress. Naderikharaji et al. (2008). Anjum et al. (2011) and Riaz et al. (2011) stated that there were decrease in stomatal conductance by increasing the moisture stress. Benlloch-Gonzalez et al. (2010) determined the effect of drought stress on stomatal closure that has been found to be dependent on nutrient in sunflower. Mehrpouyan and Nazariyan (2012) found that stress at all stages resulted in the loss of sub-stomatal CO_2 conductance of the studied cultivars.

Sub-Stomatal CO_2 conductance (µmol/m^2/sec)

The Table 4.15(a) presents the analysis of variance of canola accessions for sub-stomatal CO_2 conductance across different levels of moisture stress. Mean comparisons of canola accessions for sub-stomatal CO_2 conductance presented in Fig 4.17. The analysis of variance showed that there were significant differences in sub-stomatal CO_2 conductance of the accessions and treatments. But the interaction between accessions and treatments were non-significant. Kousar et al. (2006), Naderikharaji et al. (2008), Riaz et al. (2011) and Anjum et al. (2011) indicated the significant differences among the accessions. Sub-stomatal CO_2 conductance of the accessions ranged from 136.92 to 332.59 m mol. The accession Dunkeld showed maximum sub stomatal CO_2 conductance and significantly different from all the other accessions. Minimum sub- Stomatal conductance was observed for the accession Zn-N-6 and significantly different from the other accessions.

Table 4.15(a) Analysis of variance of canola accessions for sub-stomatal CO_2 conductance across different moisture stress levels

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatments (T)	2	24990	12495	30.16**
Accessions (A)	3	206832	68944	166.43**
T × A	6	2140	356	0.86
Error	24	9942	414	
Total	35	2439		

** = Significant at 1% probability level

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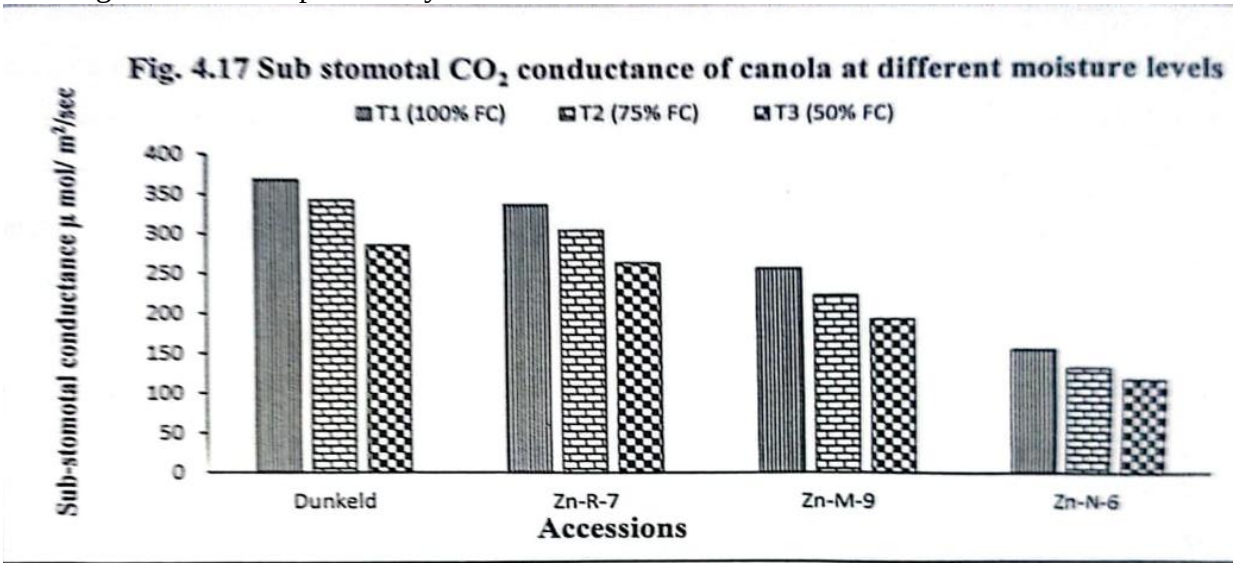
Table 4.15(b) Mean squares values from analysis of variance of canola accessions for sub- stomatal CO2 conductance under different moisture levels

Sources of variation	Degrees of freedom	Treatments		
		T_1 (100% FC)	T_1 (75% FC)	T_1 (50% FC)
Accessions	3	0.018**	0.016**	0.015**
Error	8	0.0002	0.0001	0.0009

** = Significant at 1% probability level



Mean comparisons indicated that sub- stomatal CO_2 conductance was decreased by increasing the moisture stress levels. The treatments T_1 (100% FC), T2 (75% FC) and T) (50% from each other. FC) showed 280.36, 251.58 and 215.94 µmol/m^2/sec respectively and significantly different

Mean squares values from the analysis of variance for sub-stomatal CO_2 conductance under different moisture stress levels are shown in Table 4.15(b). The accessions showed highly significant differences in all the treatments. In T1 (100% FC) the sub-stomatal CO_2 conductance ranged from 157.44 to 368.44 µmol/m^2/sec The accession Dunkeld showed the maximum sub-stomatal conductance which was significantly different form all the other accession except Zn-R-7 in T1 (100% FC). While, Zn-N-6 showed minimum sub-stomatal CO, conductance and significantly different from all the other accessions in T1 (100% FC). In T2 (75% FC) the sub-stomatal CO2 conductance ranged from 133.78 to 343.11 µmol/m^2/sec. The accession Dunkeld showed the maximum sub-stomatal conductance which was significantly different form all other accessions. Whereas, Zn-N-6 showed minimum sub-stomatal CO_2 conductance and significantly different from all the other accessions in T2 (75% FC). The sub-stomatal CO_2 conductance ranged from 119.55 to 286.22 µmol/m²/sec in T3 (50% FC). The accessions Dunkeld showed the maximum sub-stomatal CO2 conductance followed by Zn-R-7 and significantly different from all the other accessions in T3 (50% FC). While Zn-N-6 showed minimum sub-stomatal

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conductance in T3 (50% FC) and significantly different from each other. The results showed that stomatal conductance decreased by increasing moisture stress. Naderikharaji et al. (2008), Riaz et al. (2011) and Anjum et al. (2011) stated that there were decreases in CO_2, assimilation rate due to reduced stomatal conductance. Susceptible accessions showed lower sub-stomatal CO_2 conductance in all treatments of moisture to reduced stomatal conductance. Susceptible accessions showed stomatal conductance in all treatments of moisture stress. Benlloch-Gonzalez et al. (2010) mind the effect of drought stress on stomatal closure that has been found to be dependent nutrient in sunflower. Mehrpouyan and Nazariyan (2012) found that stress at all three resulted in the loss of sub-stomatal CO_2 conductance of the studied cultivars.

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