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EVALUATION OF STAND ESTABLISHMENT AND GROWTH PERFORMANCE OF WHEAT IN RESPONSE TO DIFFERENT WATER POTENTIAL LEVELS

Mohammad Akmal[1,*], Muhammad Afzal[2] and Tadashi Hirasawa[3]

[1]Department of Agronomy, University of Agriculture, Peshawar, Pakistan; [2]Department of Soil & Environmental Sciences, University of Agriculture, Peshawar, Pakistan; [3]Eco-physiology Lab, Tokyo University of Agriculture and Technology, Japan.
[*]Corresponding author's e-mail: akmal_m@hotmail.com

Early growth of a germinated wheat seed was characterized with changes in vermiculite water potentials (Ψ_W -0.03 to -1.10 MPa) and pots depth (10 to 30 cm). Deionized water was added in vermiculite for a desired Ψ_W treatment. Germinated seeds of 5-mm radicles were planted in pots and glass boxes. Experiments were conducted in controlled condition in a growth chamber. Periodic samples were taken on each sampling day to measure roots and shoot of young wheat plant. Leaf area was markedly sensitive to decrease in Ψ_W from -0.03 to -0.60 MPa with no leaf visibility at Ψ_W -1.10 MPa in 25 days old seedlings. Dry matter (DM) decreased by reducing Ψ_W, but this reduction in DM was associated to both shoot and roots losses. DM of seed-hull increased by decrease in Ψ_W. No changes in DM were observed in early 7 days growth at Ψ_W -0.03 and -0.15 MPa. Seminal roots number did not differ at higher Ψ_W but markedly decreased at low Ψ_W. The root length showed a linear reduction by decrease in Ψ_W from -0.03 to -1.10 MPa. Root branch length also shrank markedly at high (Ψ_W -0.03 to -0.06) to moderate (Ψ_W -0.60 to -1.10) decrease in Ψ_W. Root length to weight ratio was linear but negatively related to decrease in Ψ_W with a linear positive change with time after the seed transplanting. Osmotic potential and tissue moisture content declined in a linear fashion by the decrease in Ψ_W for different parts. DM of shoot and roots was observed in exponential fashion to DM of seed-hull and time to the transplanting. A linear but negative relationship was noted for shoot and roots to seed-hull fractional contribution in DM (FCDM). The study suggests marked sensitivity of root and shoot to reduction in Ψ_W in the early development stages of wheat plant. Reduction in Ψ_W markedly decreased roots and branch number and their length, which inhibited leaf initiation.

Keyword: Wheat seedlings, water potential, seminal roots, root branch, drought, osmotic potential

INTRODUCTION

Wheat (*Triticum aestivum* L.), oldest plants in cultivation by the mankind, is extensively planted worldwide due to its adaptation to a range of climates. Population growth, especially in agrarian regions will demand more production over time in the future. Limitation of available water for crops and future expected climate change might increase stress for wheat production in many arid and semi-arid regions of the world (Chen *et al*., 2012; Akmal *et al*., 2014). According to an estimate, drought may affect 99 million ha wheat in developing countries and 60 million ha in the developed countries (Rajaram, 2000). Water stress could reduce grain yield with an average loss from 17 to 70% (Nouri-Ganbalani *et al*., 2009). Although in comparison with other cereals crops e.g. maize, wheat is fairly drought tolerant (Akmal and Hirasawa, 2004). It can successfully be grown with little but well distributed precipitation in early development phase. A small quantity of rain in early wheat growth can results no loss in the grain yield. Crop failure of wheat is mostly reported when soil moisture is insufficient to complete germination and/or acute short for the initial establishment of young a wheat plant in early vegetative development phases. Once the seedling established successfully and passed from seedling to plant stage, it become auto-tropic. Its growth becomes less susceptible to environmental fluctuations while plant is able to evolve mechanism responding to a variety of environmental signals (i.e. soil moisture, light, temperature and gravity etc.). These signals can play significant role in controlling growth mechanism within the plant body. The limited water undoubtedly has a significant impact on plant growth and may cause considerable loss in productivity worldwide (Martin *et al*., 2006). Growth is results of evapotranspiration (Ca. 200-1000 times of body dry matter) during the plant life cycle (Hasiao and Xu, 2000). It mainly keeps leaf open for adequate CO_2 exchange to build photo- assimilates for body maintenance. Under deficient water condition, both leaf and shoot growth is inhibited (Nonami and Boyer, 1990; Chazen and Neumann, 1994) and/or roots elongation is continued even under complete inhibition of shoot (Westgate and Boyer, 1985; Spollen *et al*., 1993). It may be possible that

roots play a key role in water uptake regulation and maintaining balance of plant water budget (Javot and Maurel, 2002). Nonetheless, regulation of roots water flow properties is still not fully identified at lower water potentials (Aroca *et al*., 2012). We have noted that length of a seminal root of wheat declined at reduction of the substrate moisture content. This decrease in root length may be due to decrease extensibility and/or increase in yield threshold of roots' cell wall or may be due to reduction in hydraulic conductivity of root tissue (Akmal and Hirasawa, 2004).

Soil moisture fluctuation affect has been documented very frequently and in detail on plants shoot growth. However, limited information is available on root-growth relationship under the changing moisture on crop early establishment (Saidi *et al*., 2010). Despite roots study is equally important while it exploits resources (e.g. nutrients and water) for plant growth and maintenance. Realization of high productivity, therefore, depends on adequate partitioning of carbon to root growth for efficient utilization of soil resources (Farooq *et al*., 2009). At low soil moisture content, adverse effects can be observed on shoot growth and its functions (Gazal and Kubiske, 2004; Hirasawa *et al.*, 1994; Akmal and Hirasawa, 2004). Relatively less sensitivity of roots than shoot under drought is advantageous to plants under drying soil for initial establishment, whereas, young seedling is more vulnerable to drying soil-surface layers. Root growth potential is simple physiological attribute used for measuring seedling quality in a deficit soil moisture condition (Gazal *et al*., 2004).

Literature on roots and shoot growth under decreasing Ψ_W is limited, sometime may also contradicts for roots and shoot growth responses with decreasing Ψ_W. Moreover, drought is becoming an issue for future agriculture production in most parts of the world. Climate change by increased in CO_2 may contribute in rise of average air temperature, which may cause water shortage for crop like wheat famous as a rain-fed crop (Saidi *et al.*, 2010). It is known that water shortage to plants has resulted economic losses in many regions (Yin *et al*., 2005). This study, therefore, focused on root growth of wheat seminal roots and their branch behavior in early establishment phases by decreasing in the vermiculite water potentials. In this stage conversion of a seedling to plant is highly critical for survival in field in collaboration with decrease in temperature for the following days to sustain staple food for the future population.

MATERIAL AMD MATHODS

***Plant materials and treatments*:** Seeds of wheat cv. Bandowase were used for growth in the vermiculite for all experiments conducted at Eco-physiology Lab, Tokyo University of Agriculture and Technology, Japan. Firstly, seeds were washed with 0.25% NaOCl solution for 10 min, rinsed with de-ionized water for a while and placed on wet filter papers in a glass Petri-dish in incubator. The seeds were allowed to germinate at 25°C in dark in an incubator for 30 h. Six germinated seeds of 5-mm radicles length were planted in plastic pots (10.6 x 30 cm) that had already been filled with vermiculite having different water potentials (Ψ_W). The bottom-end of plastic tube (pot) was sealed a day before filling with 0.5 cm thick card duly wrapped tightly in a two-fold aluminum foil-film. The aluminum foil-film was used to avoid moisture absorbance from treatment and/or protect material inside from outer contaminations during the study. To avoid evaporation from pots, upper end was also sealed tightly with a 0.5 cm thick Styrofoam cover made for with having holes in it to place the germinated seeds for growth in pots. Seedlings after transplanting were allowed to grow in an incubator under the controlled environmental conditions at ±23/17°C, 250 µ mol photon m^{-2} S^{-1} (PAR), 60/80% relative humidity and a photoperiod of 12 h for all experiments.

Experiment 1 was conducted in June, using four Ψ_W treatments (-0.03, -0.15, -0.60 and -1.10 MPa) to study wheat development for 25 days growth after germination (Fig. 1 to 7). Experiment 2 was conducted in July, using four Ψ_W treatments (-0.03, -0.07, -0.10 and -0.15 MPa) to study high Ψ_W response on plant growth for 7 days after germination (Table 1). Experiment 3 was conducted in August, using two Ψ_W treatments (-0.03 and -0.15 MPa) and three depths (10, 20, and 30 cm) in response to observe roots and branch growth behavior of wheat plant for 13 days after germination (Table 2). Experiment 4 was conducted in September, using four Ψ_W treatments (-0.03, -0.15, -0.60 and -1.10 MPa) focusing moisture and solute content in wheat plant's parts (i.e. seed-hull, roots and shoot) for 7 days growth after germination. Experiment 1-3 were done in pots as explained earlier while Exp. 4 was conducted in special glass-boxes (700 x 140 x 40 mm) prepared for the study. For Ψ_w treatments, vermiculite from Fukushima, Japan was sieved through 2-mm mesh, initially moist with a half strength Hoagland's solution (Hoagland and Arnon, 1950) to achieve desired the lowest Ψ_w (-1.10 MPa). The de-ionized water of known quantity was added in containers for any subsequent higher Ψ_w treatments (-0.60, -0.15 and -0.03 MPa). The 10^{-4} M $CaCl_2$ was also equally added in solution to extend water-holding capacity of vermiculite for the study. The sealed containers of different Ψ_w treatments were regularly shaken periodically for six days to homogenize substrate moisture content. On the day of transplanting, all pots were first filled with desired vermiculite treatments of known quantity and immediately sealed from top face with a styro-foam cover. Six germinated seeds were placed in holes of each tube and subsequently covered with a centimeter thick layer of the vermiculite of similar Ψ_w. Seedlings were allowed to grow in growth chamber for the study period.

Table 1. Growth performance of seven days old wheat seedlings to varying water potential levels.

	Vermiculite Ψ_w (MPa)			
Parameters (plant^{-1})	**-0.03**	**-0.07**	**-0.11**	**-0.15**
Leaf area (cm^2)	3.37 a	3.11 ab	2.85 ab	2.36 b
FCLA 1 (%)	84.64 a	87.08 a	87.43 a	91.24 a
FCLA 2 (%)	15.36 a	12.92 a	12.58 a	8.76 a
Plant DM (mg)	34.27 a	33.54 a	34.46 a	31.79 a
Seed-hull DM (mg)	15.59 a	14.69 a	16.59 a	15.13 a
Shoot DM (mg)	11.09 a	10.84 a	10.44 a	9.58 a
Roots DM (mg)	22.09 a	23.97 a	21.55 a	22.29 a
Root number	5.22 a	5.22 a	5.22 a	5.11 a
Root length (mm)	600.30 ab	634.50 a	632.80 ab	546.90 b
Branch number	11.11 a	8.56 ab	7.89 ab	4.67 b
Branch length (mm)	59.17 a	49.18 a	39.16 a	20.10 a
Roots & Branch length (mm)	659.50 ab	683.70 a	671.90 ab	567.00 b
RLWR (mm mg^{-1})	87.33 a	85.60 a	91.45 a	80.51 a
Roots volume (ml)	0.99 a	0.88 ab	0.79 ab	0.72 b
Roots to shoot ratio	0.69 a	0.74 a	0.71 a	0.74 a

RLWR = Root length to weight ratio; Mean with a common letter in a row are not significant, Tukey's studentized range (HSD) test ($p \leq 0.05$).

Table 2. Water potential levels and pot depths response on growth performance of 13 days old wheat plant in pots.

	Days (d) after germination (n = 18)		**Ψ_w (MPa) (n = 18)**		**Pot's depth (D) (n =12)**			**Interactions**	
Parameters (plant^{-1})	**07**	**13**	**-0.03**	**-0.15**	**10**	**20**	**30**	**$\Psi_w$xd**	**Ψ_wxD**
Leaf area (cm^2)	2.68b	6.07a	5.99a	2.74b	4.29a	4.27a	4.47a	**	NS
FCLA 1 (%)	52.90b	84.94a	64.24b	73.61a	68.65a	68.67a	69.45a	**	NS
FCLA 2 (%)	15.06b	44.90a	34.33a	25.52b	30.03a	30.07a	29.68a	**	NS
Plant DM (mg)	31.76b	40.20a	37.75a	34.20b	36.11a	36.42a	35.40a	**	NS
Seed-hull DM (mg)	16.64a	6.60b	8.82a	14.41a	12.23a	12.00a	10.61a	NS	NS
Shoot DM (mg)	8.80b	17.00a	15.39a	10.41b	12.93a	12.90a	12.87a	*	NS
Roots DM (mg)	6.31b	16.59a	13.54a	9.37b	10.94a	11.51a	11.92a	**	NS
FCDM-Seed-hull (%)	52.04a	17.22b	26.12a	43.14b	36.26a	35.07a	32.55a	NS	NS
FCDM-Shoot (%)	27.95b	42.00a	39.86a	30.09b	34.92a	34.76a	35.23a	*	NS
FCDM-Roots (%)	20.02b	40.78a	34.02a	26.78b	28.82b	30.2ab	32.20a	NS	NS
Root number	4.70a	4.78a	4.82a	4.76a	4.75a	4.67a	4.81a	NS	NS
Root length (mm)	560.10b	1068.00a	979.30a	648.20b	715.00b	849.00a	878.00a	NS	*
Branch number	10.35b	1481.00a	65.80a	23.08b	40.20a	45.80a	47.30a	**	*
Branch length (mm)	64.70b	1481.00a	1270.00a	275.00b	780.00a	765.00a	772.00a	**	NS
Roots -Branch length (mm)	625.00b	2548.00a	2250.00a	923.00b	1495.00a	1614.00a	1650.00a	**	NS
RLWR (mm mg^{-1})	96.00b	146.70a	150.40a	92.33b	118.40a	122.90a	122.80a	*	NS
Roots volume (ml)	0.91b	2.48a	2.28a	1.11b	1.69a	1.68a	1.72a	**	NS
RSR	0.73b	0.97a	0.83b	0.87a	0.81a	0.85a	0.90a	*	NS

Mean with a common letter within category in a row are not significant; Tukey's studentized range (HSD) test ($p \leq 0.05$) * ($p \leq 0.001$) and NS = Non-significant.

For Experiment 3, same pots were tightly filled with dry vermiculite at marked depth and sealed with a hard plastic cover duly wrapped in twofold water resistant sheets to avoid any kind of roots or moisture infiltration form growth zone. For experiment 4, two germinated seeds were placed in a box and immediately covered with free glass cover to study the Ψ_w responses for moisture and solute potentials (Ψ_o) in wheat plant parts. All boxes were placed inclined at 50° from ground in a growth chamber. Desired water potential for a treatment of this study was achieved by adding known volume of de-ionized water in known quantity of vermiculite. We have already established a

relationship for vermiculite water potential by regressing its moisture and Ψ for treatments (Akmal and Hirasawa, 2004).

***Dry matter and leaf area measurements*:** Samples of moist vermiculite were collected at start and at every harvest day from 10 and 20 cm depths from tubes and subsequently oven dried at 105°C for about 72 h to determine changes in Ψ_w. A slight reduction in Ψ_w of vermiculite during study at a stable rate was observed for all treatments with time after transplanting. In first experiment, four periodic measurements were taken at six days intervals. First sampling was purposely delayed for a day to allow germinated seeds to adjust with varying Ψ_w treatments. On each sampling day, three uniform plants pot^{-1} were selected and examined. Three pots for each treatment were harvested on a sampling day representing three repeats. On a sampling day, bottom and top faces of pots were carefully opened avoiding any kind of disturbances to young plants. Plants with vermiculite stick to roots collected in a tray and washed with running tap water. During washing, all roots were carefully cleaned and separated. Three plants uniforms in appearance were selected from a pot for measurements. Roots and shoot were carefully removed from seed-hull (rest of the seed after emergence). All plant parts i.e. shoot, roots, and seed-hull were preserved in soft moist tissues for measurements. Leaf area of each plant was determined with the help of leaf area measuring's machine (AAM-9, Hayashi Denko, Tokyo, Japan). Fractional contribution of leaf area (FCLA) for leaf at nodal po
sition 1 and 2 of the total leaf area was calculated as ratio of the respective leaf in total plant leaf area on a respective sampling day. Dry matter (DM) was determined by oven drying samples at 80°C for not less than 48 h. Oven dried samples of shoots, roots and seed-hulls were separately weighed on an analytical balance. Likewise, fractional contribution of dry matter (FCDM) in total mass of plant organs was estimated as ratio of the respective parts dry matter in total plant DM in percent.

***Root number, length and volume measurements*:** To measure the seminal root number, braches and their length, all roots of a treatment were arranged in a plastic tray, which was already bedecked with six-ply soft tissues-sheets covered with an addition two-ply black polyester fiber sheet (BDK, Yunichika, Tokyo, Japan). The material in tray was retained fully moist with de-ionized water facilitating individual root and branch segregation through water for further measurements. On arranging all roots and branches of a treatment in proper order, photographs were taken and roots materials were collected in labeled bags for further measurement of dry matter. Roots photographs were subsequently analyzed through a computer software image analysis program (SigmaScan, Jandel Scientific Software, USA) for root length, branch length and their number. Average root and branch lengths were estimated. The root to shoot ratio (RSR) was derived by dividing weight of roots on shoot excluding seed-hull. Roots volume was measured for rest of the three plants of a tube. Clean roots were immersed in known quantity of water in a graduated beaker and displacement of water was measured as root volume for a treatment. Roots, shoots and seed-hull samples were separately oven dried in bags at 80°C to determine their dry mass.

***Moisture content and solute concentration*:** On sampling day, open face of the glass box was carefully removed in a moisture- saturated chamber (Akmal and Hirasawa, 2004). Roots were excised with a sharp razor from shoot and seed-hull. All three parts of a plant were separately collected in an airtight glass bottles working within a humid chamber. The part's moisture contents were determined by subtracting differences in fresh matter of a plant parts and oven dried at 80°C for not less than 48 h. For measurements of the solute concentration (Ψ_o), samples of plant parts were immediately frozen in liquid N and stored in a freezer at -80°C. On the day of measuring Ψ_o of plant parts, samples were de-frozen at room temperature (25°C) for about 30 min. The solute potential was determined directly by placing each plant part in a separate chamber of a thermocouples psychrometer (Akmal and Hirasawa, 2004). Osmotic potential of plant parts (i.e. roots, shoot and seed-hull) was measured by an isopiestic technique with a thermocouple psychrometer (Boyer and Knipling, 1965).

***Statistical analyses*:** For comparing differences in plant parts, leaf area, roots and branch number and length, roots shoot ratio and their interactive effects, we calculated a combined ANOVA in SAS (SAS Institute, 2008). Tukey's studentized range (HSD) test was used to compare responses of the treatments ($p \leq 0.05$).

RESULTS

***Leaf area*:** Leaf area (cm^2) of wheat plants was significantly decreased with the decrease in water potential (Fig. 1). Highest leaf area was recorded at Ψ_w -0.03 MPa, followed by with a significant ($p \leq 0.001$) decrease in Ψ_w -0.15 and Ψ_w -0.60 MPa (Fig. 1a). Regarding subsequent plant samplings with age, the leaf area was increased linearly between 7 and 19 d after transplanting with a slight increased from 19 to 25 d after transplanting (Fig. 1b). Interactive effect of varying Ψ_w and sampling interval showed the highest leaf area for Ψ_w -0.03, followed by Ψ_w -0.15 MPa with a significant increase for every subsequent sampling with similar fashion but far lower values for Ψ_w -0.60 MPa (Fig. 1c). Total leaf area of Ψ_w -0.03 and -0.15 MPa increased moderately in a linear fashion between 7^{th} and 19^{th} d after the transplanting but with a relatively slower rate thereafter from 19^{th} to 25^{th} d after transplanting. Treatment Ψ_w -0.60 MPa showed a small fraction of the total leaf area on 19^{th} d after transplanting with

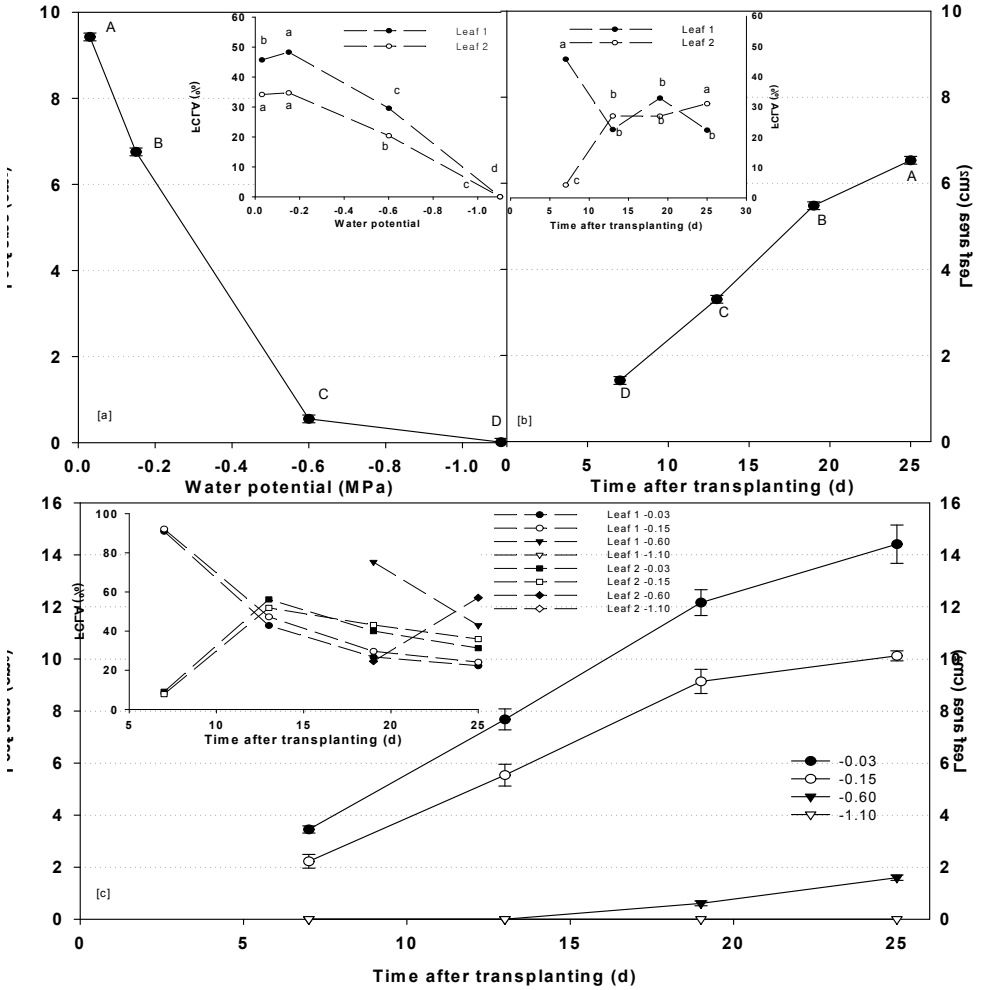

Figure 1. Different water potentials effect on leaf area (cm^2) of a young wheat plant [a] for 25 d after transplanting a germinated seed [b]. The interaction of treatments Ψw x time after transplanting is also shown [c]. The inset figures show fraction of total leaf area (FCLA) for leaf 1 and 2. Letters (a, b & c) indicate statistically significance (p≤0.001) difference using HSD test.

an almost linear increment on 25th d after transplanting. The lowest Ψ_w -1.10 MPa was unable to show any leaf area in 25 d growth after the transplanting. Moderate reduction in vermiculite Ψ_w -0.03 and -0.015 MPa (Table 1) or pot depths (10 to 30 cm) did not show any significant (p≤0.05) changes in wheat plant leaf area (Table 2). **I**nset figures in box (Fig. 1a) showed fraction of total leaf area (FCLA) for leaf 1 and 2. Treatment Ψ_w -0.15 MPa showed the highest (p≤0.001) FCLA for leaf 1, followed by slight (Ψ_w -0.03 MPa) to moderate (Ψ_w -0.06 MPa) decrease approaching to zero (Ψ_w -1.10 MPa). The FCLA for leaf 2 was non-significant (p≤0.05) for treatment high Ψ_w (-0.03 and -0.15 MPa) but decreased for medium Ψ_w (-0.60 MPa) with zero at the lowest Ψ_w (-1.10 MPa). While averaged across Ψ_w, FCLA for leaf 1 was the highest at 7th d after transplanting and significantly (p≤0.001) decreased at 13th d after the transplanting. FCLA remained unchanged (p≤0.05) from 13th to 19th d after the transplanting with a significant decreased at 25th d (Box Fig. 1b). The FCLA for leaf 2 was lowest at 7th d after transplanting, increased (p≤0.05) at 13th with no further change at 19th d after transplanting. It was recorded the maximum (p≤0.05) at 25th d after transplanting. Interaction (Ψ_w x samplings) showed a reduction in FCLA for leaf 1 which was strong (7th and 13th) to moderate (13th to 25th) for Ψ_w -0.03 and -0.15 MPa (Box in Fig. 1c). FCLA for leaf 2 increased (7th to 13th) with a trivial decrease (13th to 25th) for Ψ_w -0.03 and -0.15 MPa. FCLA for Ψ_w -0.60 MPa was observed on 19th d after transplanting with decrease (p≤0.05) for leaf 1 and increase (p≤0.05) for leaf 2 from 19th to 25th d after transplanting. FCLA for leaf 1 and 2 did not influence with changes in Ψ_w (Table 1) or depths (Table 2).

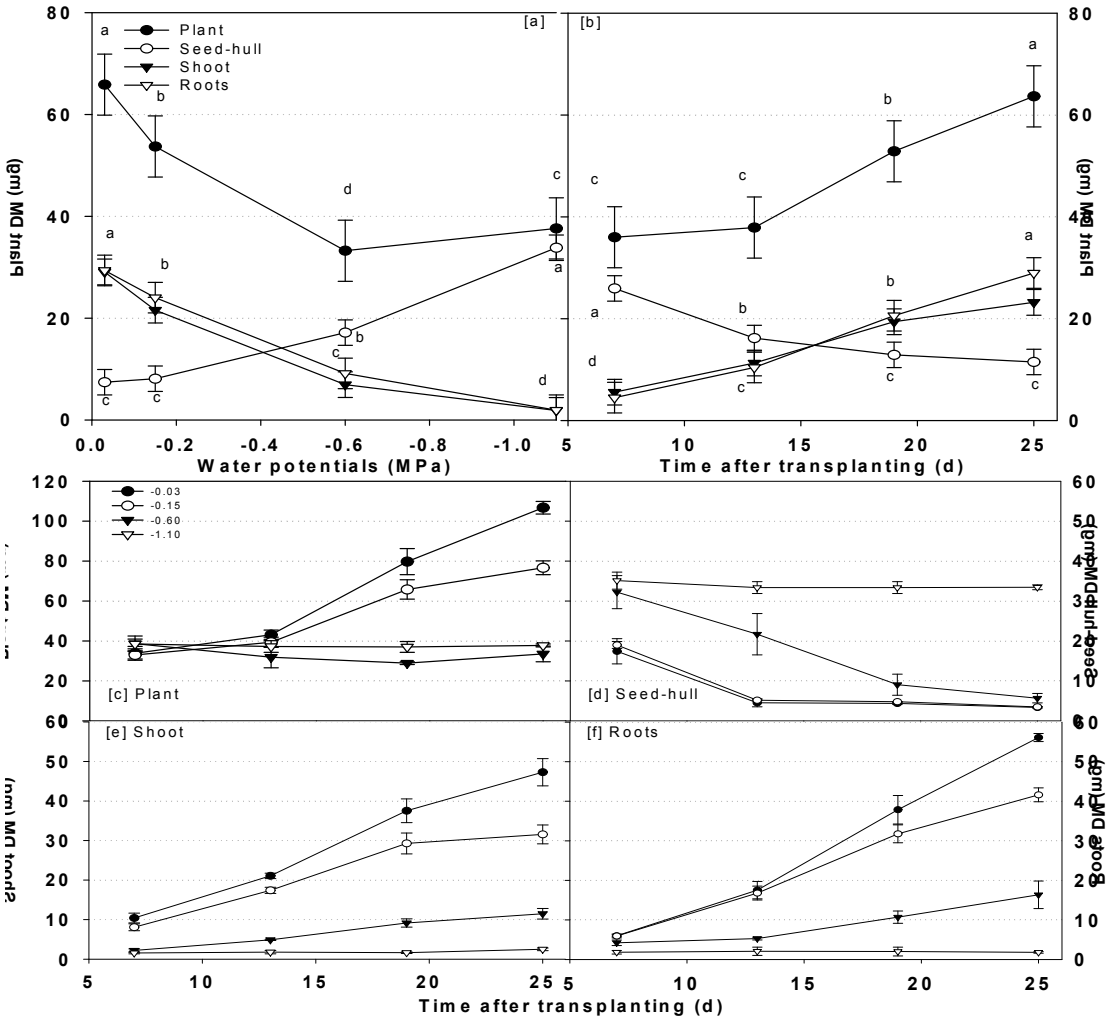

Figure 2. Different water potentials effect on dry matter (mg) of young wheat plant and parts [a] for 25 d growth after transplanting a germinated seed [b]. The interactions of treatment Ψ_w x time after transplanting are also shown [c] for total plant, seed-hull, shoot and roots in separate boxes. Letters (a, b c, & d) indicate statistically significance (p≤0.001) difference using HSD test.

***Dry mater (DM)*:** Maximum plant DM was observed at Ψ_w -0.03 MPa, which significantly (p≤0.05) decreased by decreasing Ψ_w -0.15 and -1.10 MPa (Fig. 2a). The minimum plant DM was observed for Ψ_w -0.06 MPa. Total plant DM did not change (p≤0.05) with minor changes in Ψ_w from -0.03 to -0.15 MPa (Table 1) or altering pot depth from 10 to 30 cm (Table 2). For individual plant parts, the DM of a seed-hull increased by reducing Ψ_w from -0.03 to -1.10 MPa. Seed hull DM for Ψ_w -0.03 and -0.15 MPa was lower but did not differ (p≤0.05) from each other, showed a significant increase for Ψ_w -0.60 with a further significant increase for Ψ_w -1.10 MPa. Contrary to the seed hull DM, plant shoot and roots DM decreased (p≤0.001) with a comparable fashion by decreasing Ψ_w from -0.03 to -1.10 MPa. However, slight changes in Ψ_w from -0.03 to -0.15 MPa (Table 1) or in pot's depth (10 to 30 cm) did not show any statistical differences in shoot and/or roots DM (Table 2). Total plant DM did not change (p≤0.05) between 7 and 13 d after transplanting, but did increase significant (p≤0.05) at 19 and 25 d after transplanting (Fig. 3b). Seed hull DM decreased (p≤0.05) between 7 and 19 d with a non-significant changes thereafter between 19 and 25 d after transplanting. Both roots and shoot DM showed a significant (p≤0.05) increment for every subsequent sampling from 13 to 25 d after transplanting. Moderate changes in Ψ_w did not show significant (p≤0.05) effect in seed hull, shoot and roots DM (Table 1) or by changing pot's depth (Table 2). Interaction (Ψ_w x samplings) showed highest plant DM for Ψ_w -0.03, followed by -0.15, -0.60 and -1.10 MPa (Fig. 2c). Averaged across Ψ_w, plant DM remained almost stable (p≤0.05) for 7 and 13 d. with linear fashion growth for 13 and 25 d after transplanting for all

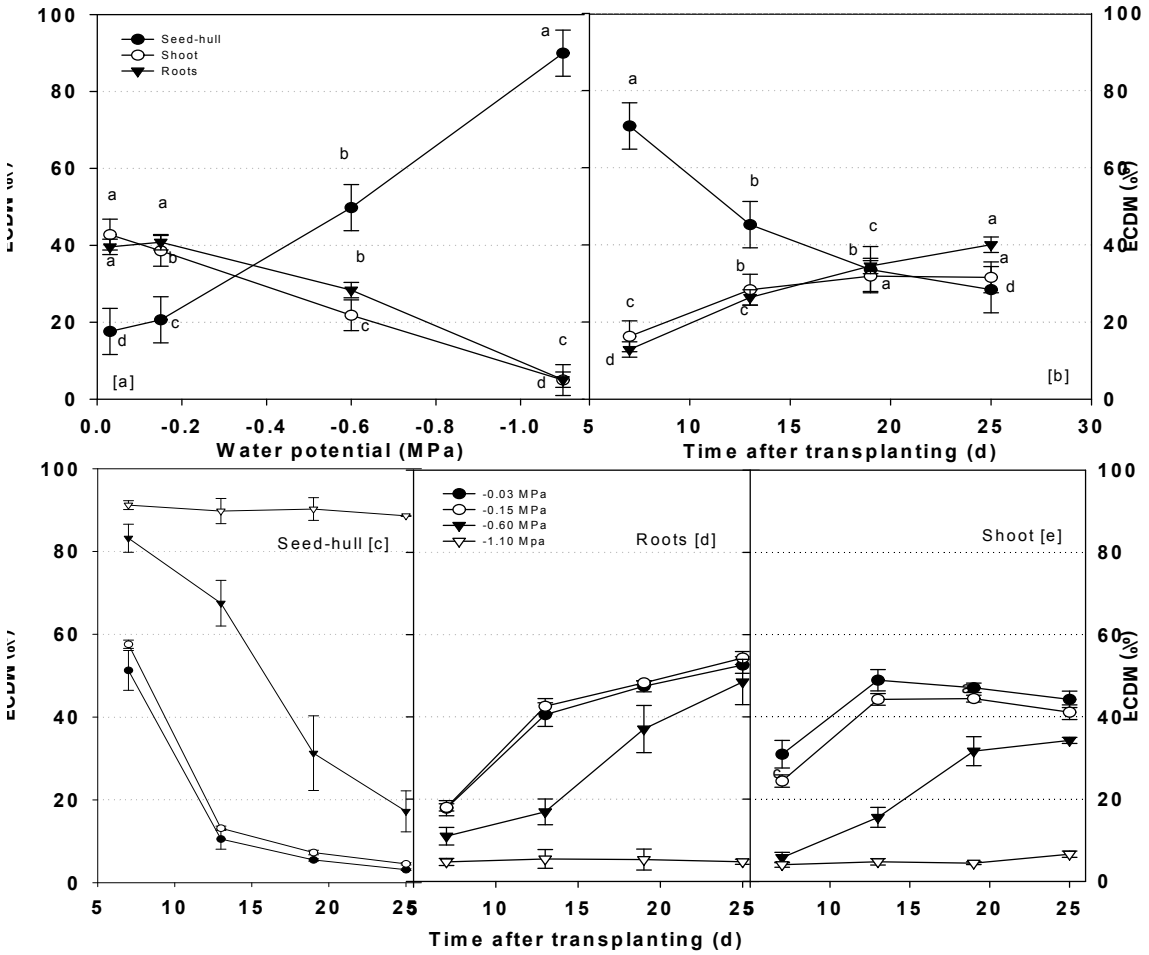

Figure 3. Different water potentials effect on plant parts fractional contribution in total dry matter (FCDM) of a young wheat plant [a] for 25 d growth after transplanting a germinated seed [b]. The interactions of treatment Ψ_w x time after transplanting is also shown [c] for seed-hull [c1], shoot [c2] and roots [c3]. Letters (a, b & c) indicate statistically significance ($p \leq 0.001$) difference using HSD test.

Ψ_w which can be expressed with slopes of regressions i.e. 2.84, 2.03, and 0.70 ($r^2 = 0.99$) for Ψ_w -0.03, -0.15, and -0.60 MPa, respectively. Seed-hull DM remained almost stable for Ψ_w -1.10 MPa from 7 to 25 d after transplanting (Fig. 2d). It decreased at a stable rate for Ψ_w -0.60 MPa from 7 to 19 with no change ($p \leq 0.05$) from 19 to 25 d after transplanting. Seed hull DM for Ψ_w -0.15 and -0.03 MPa were non-significant ($p \leq 0.05$). It was initially decreased (7 and 13 d) and then remained stable (13 to 25 d) after transplanting. Shoot DM was maximum at Ψ_w -0.03 MPa and significantly ($p \leq 0.05$) increased at every subsequent sampling date, followed by Ψ_w -0.15 MPa with marked to moderate increases in every subsequent samplings as compared to Ψ_w -0.03 MPa (Fig. 2e). Shoot DM in Ψ_w -0.60 MPa also increased but with far lower values from Ψ_w -0.15 MPa from 7 and 25 d after transplanting. Shoot DM of Ψ_w -1.10 MPa remained almost constant for 7 to 25 d after transplanting. Likewise, roots DM of Ψ_w -0.03 and -0.15 MPa increased in a similarly fashion for 7 and 13 d after transplanting, differed moderate at 19 to marked at 25 d after the transplanting (Fig. 2f). Root DM was lower for Ψ_w -0.60 MPa at 13 d after transplanting with a linear increase from 13 to 25 d after transplanting. Root DM in Ψ_w -1.10 MPa was almost constant for 25 d growth. Fractional contributions of parts dry matter in total plant dry matter (FCDM) showed mild (Ψ_w -0.03 to -0.15 MPa) to marked (Ψ_w -0.60 to -1.10 MPa) increases in present study (Fig. 3a). Seed hull FCDM under moderate reduction in Ψ_w did not vary (Table 1). Likewise it did not influence by altering pot depths (Table 2). Contrary to the seed hull, Shoot and root FCDM decreased with decrease in Ψ_w. A consistent reduction ($p \leq 0.05$) in shoot FCDM was recorded for Ψ_w -0.03 to -1.10 MPa but roots did not show changes in Ψ_w -0.03 and -0.15 MPa. FCDM decreased ($p \leq 0.05$) thereafter for each reduction in Ψ_w. Shoot and roots FCDM at moderate reductions in Ψ_w

was not influenced (Table 1) but roots FCDM decreased ($p \leq 0.05$) when pot-depth limited from 30 to 10 cm (Table 2). Regardless of Ψ_w, seed-hull FCDM decreased ($p \leq 0.05$) for each subsequent sampling (7 to 25 d) after transplanting (Fig. 4b). Shoot FCDM showed increment ($p \leq 0.05$) from 7 to 19 d after transplanting and remained non-significant thereafter. Nonetheless, roots FCDM showed increments (7 to 25 d) after transplanting. Interaction (Ψ_w x samplings) revealed marked decreases in seed-hull FCDM (7 to 13 d) for Ψ_w -0.03 and -0.15 MPa, with further minor losses (19 and 25 d) after transplanting (Fig. 4c). FCDM for seed hull (Ψ_w -0.60 MPa) was obviously declined for each sampling (7 to 25 d) after transplanting but at slow rates than the Ψ_w -0.03 & -0.15 MPa. Negligible loss observed in seed-hull FCDM from 7 to 25 d after transplanting for Ψ_w -1.10 MPa. Roots FCDM increased moderate (7 to 13 d) to marked (13 to 25 d) for Ψ_w -0.03 and -0.15 MPa (Fig. 3d). Roots FCDM for Ψ_w -0.60 MPa was lower that Ψ_w -0.03 and -0.15 MPa but showed mild (7-13 d) to marked (13 to 25 d) increases after the transplanting. Roots FCDM at Ψ_w -1.10 MPa remained almost stable for 25 d growth after transplanting. Shoot FCDM increased (7 to 13 d) at Ψ_w -0.03 than -0.15 MPa in first week of transplanting (7 to 13 d) with a slight reduction thereafter (13 to 25 d) with slightly higher readings for Ψ_w -0.03 than -0.15 MPa (Fig. 3e). Shoot FCDM for Ψ_w -0.60 MPa showed marked increment (7 to 19 d) after transplanting with almost stable rates for 19 to 25 d after transplanting. Shoot FCDM for Ψ_w -1.10 MPa was observed almost stable for 25 d growth after the transplanting.

***Root length and number*:** The seminal root length (mm) and number under Ψ_w treatments is shown in Figure 4. A strong (Ψ_w -0.03 to -0.60 MPa) to moderate (Ψ_w -0.60 to -1.10 MPa) reduction occurred in the root length when Ψ_w reduced (Fig. 4a). By averaging across Ψ_w treatments, roots length showed consistent significant increases from 7 to 25 d after transplanting (Fig. 4b). Moderate reduction in treatments Ψ_w showed no change in roots length for -0.03 to -0.11 MPa, however, root length of -0.11 and -0.15 MPa also did not differ statistically (Table 1). Similarly, by decreasing pot length from 30 to 20 cm did not show any change ($p \leq 0.05$) in root length but did reduce root length thereafter from 20 to 10 cm depth (Table 2). Interactive effect of treatments (Ψ_w x samplings) revealed the highest roots length for Ψ_w -0.03 MPa with a consistent marked increment on every next samplings (Fig. 4c). Treatment Ψ_w -0.15 MPa did not differ

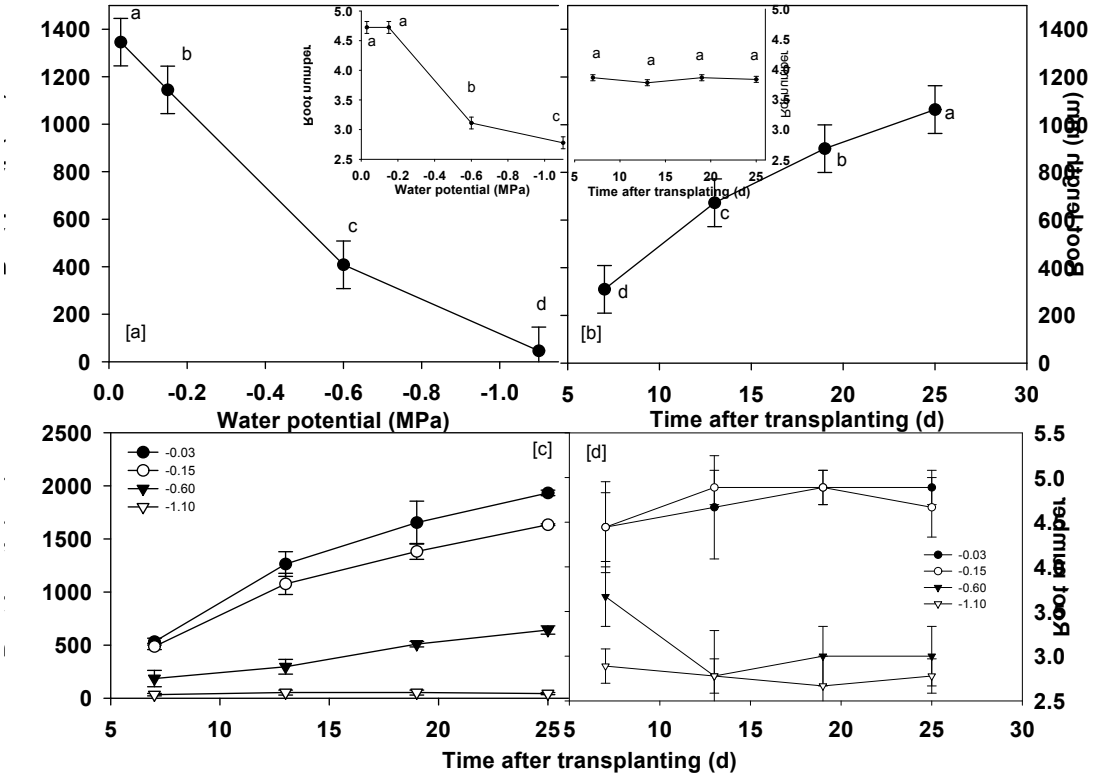

Figure 4. Different water potentials effect on root length (mm) of a young wheat plant [a] for 25 d growth after transplanting a germinated seed [b]. Interactions of treatments Ψw x time after transplanting is also shown [c]. The inset figures show root number and the interactions showed separately [d]. Letters (a, b & c) indicate statistically significance ($p \leq 0.001$) difference using HSD test.

than Ψ_w -0.03 MPa within 7 d after transplanting but did show a substantive increase for every next sampling. However, differences between Ψ_w -0.03 and -0.15 MPa extended wider from 13 to 25 d after transplanting. Nonetheless, root length in Ψ_w - 0.60 MPa was recorded far below than Ψ_w -0.15 MPa for samplings after transplanting. Root length of treatment Ψ_w -1.10 MPa remained stable for all samplings for 25 d after transplanting. The inset Figure 4 shows root number of wheat plant. The root number at Ψ_w -0.03 and -0.15 MPa did not differ statistically; however, declined considerably for Ψ_w -0.06 and -1.10 MPa (Box in Fig. 4a). By averaging across Ψ_w treatments, root number did not change between 7 and 25 d after transplanting (Box in Fig. 4b). Interaction of treatments (Ψ_w x samplings) showed higher root number for the high (Ψ_w -0.03 and -0.15) than low (Ψ_w -0.60 and -1.10) MPa for all samplings (Fig. 4d). However, root number did not show any change ($p \leq 0.05$) between the high and low Ψ_w from 13 to 25 d after transplanting. Root number did not show any change under the moderate reduction in Ψ_w (Table 1) and/or altering pot depth (Table 2).

***Branch length and number*:** Branch length (mm) of wheat plant seminal roots (BL) is shown in Fig. 5 and their number in the inset boxes. The BL showed a marked ($p \leq 0.001$) decrease when vermiculite Ψ_w reduced from -0.03 to -1.10 MPa (Fig. 5a). By averaging across Ψ_w, BL showed a modest to marked increments for 7 to 13 and 13 to 25 d after transplanting, respectively (Fig. 5b). Moderate reduction in Ψ_w -0.03 and -0.15 MPa did not show ($p \leq 0.05$) any changes in BL (Table 1). Similarly a non-significant effect in BL was noted for different pot depths (Table 2). The interactive effect of treatments (Ψ_w x samplings) showed moderate increase in BL for all Ψ_w treatments with higher for Ψ_w -0.03, followed by -0.15, with lowest for -0.06 MPa between

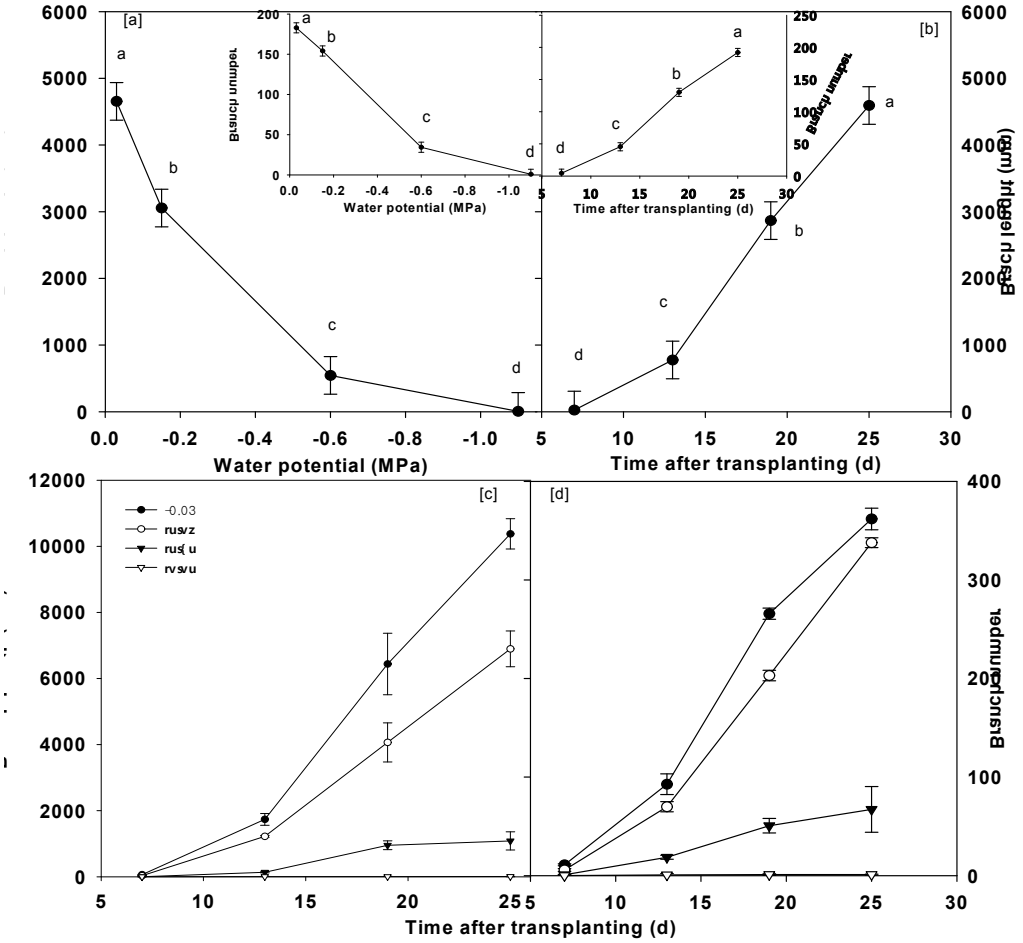

Figure 5. Different water potentials effect on branches length (mm) of a young wheat plant [a] for 25 d after transplanting a germinated seed [b]. The interactions of Ψw and time after transplanting are also shown [c]. The inset figures show root number and interactions are shown separately [d]. Letters (a, b & c) indicate statistically significance ($p \leq 0.001$) difference using HSD test.

7 and 13 d after the transplanting (Fig. 5c). However, marked increments were noted in BL for every next sampling on 19 and subsequently on 25 d after transplanting with much higher readings for Ψ_w -0.03, followed by Ψ_w -0.15 and far lower than Ψ_w -0.15 MPa for treatment Ψ_w -0.60 MPa. Treatment Ψ_w -1.10 did not show any BL in 25 d after transplanting. The branch number declined linearly between Ψ_w -0.03 and -0.60 MPa (Box in Fig. 5a). Root branch number significantly increased for every subsequent sampling (Box in Fig. 5b). Treatment Ψ_w -1.10 MPa did not show any branch number on root. Moderate reduction in Ψ_w between -0.03 and -0.11 MPa (Table 1) and variations in pot depth from 30 to 10 cm (Table 2) did not show any changes in seminal root branch number. Root branch number with time showed almost linear trends for different Ψ_w treatments (Fig. 5d). A significant moderate to marked increases were observed in branch number of Ψ_w -0.03 and -0.15 MPa for 7 to 13 and 13 to 25 d after transplanting respectively. Contrary to that, Ψ_w -0.60 MPa showed relatively a stable increment for all four samplings during 7 and 25 d after the transplanting.

***Total root length and RLWR*:** Total roots length (mm) including branches (TRL) to their weight (mg) ratio (RLWR) under different Ψ_w is expressed in Fig. 6. The TRL markedly decreased when Ψ_w dropped from -0.03 to -0.60 MPa approaching close to zero for Ψ_w -1.10 MPa (Fig. 6a). Irrespective of Ψ_w treatments, almost a linear increased ($p \leq 0.001$) was seen in TRL from 7 to 25 d after the transplanting (Fig. 6b). Slope of increment was 304.71 (mm) with a strong positive relationship ($r^2 = 0.98$). TRL did not influence under moderate reduction in Ψ_w -0.03 and -0.11 but did decrease in Ψ_w -0.15 MPa (Table 1). Pot depth did

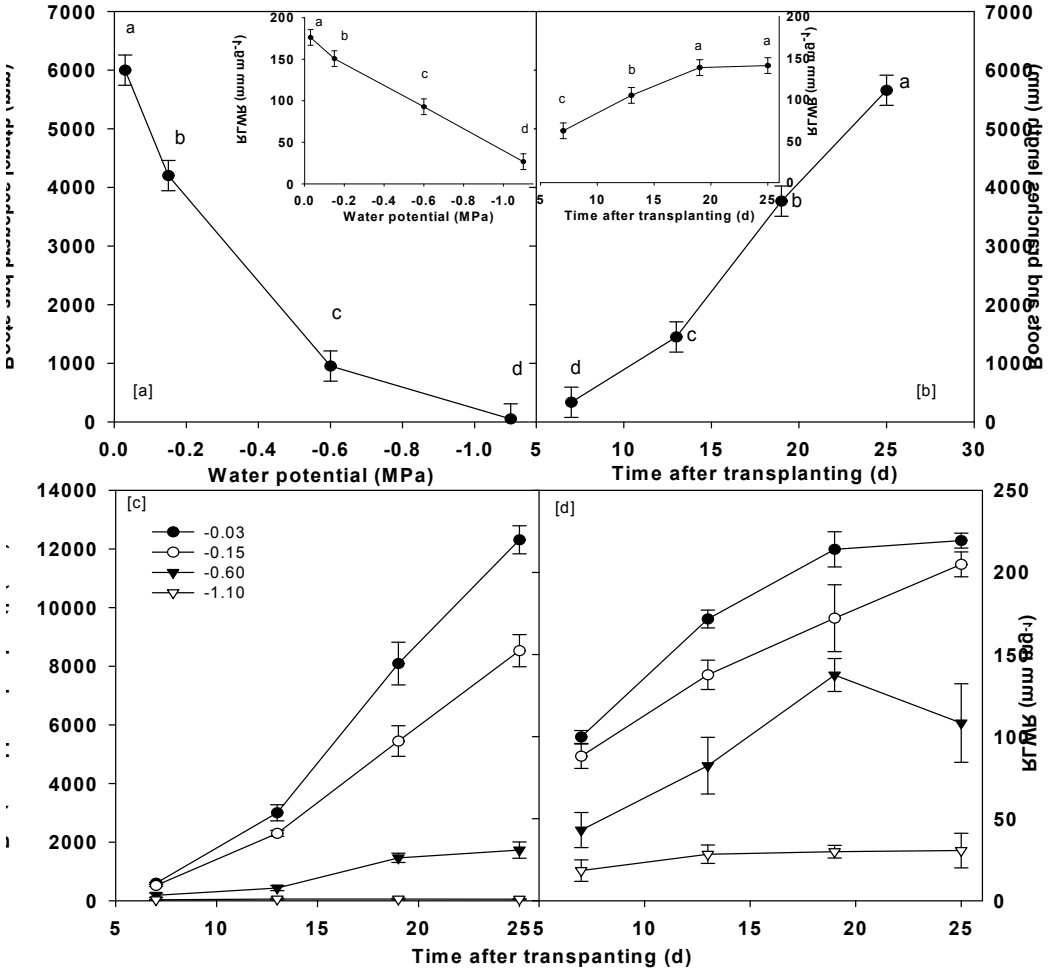

Figure 6. Different water potentials effects on total length of roots and braches (mm) of a young wheat plant [a] for 25 days after transplanting a germinated seed [b]. The interactions of Ψw and time after transplanting are also shown [c]. The inset figures show root number and interactions are shown separately [d]. Letters (a, b & c) indicate statistically significance ($p \leq 0.001$) difference using HSD test.

not show any significant difference in TRL (Table 2). The interactive effect of treatments revealed relatively stable (7 to 13 d) to marked increments in TRL for 13 to 25 d after the transplanting (Fig. 6c). Rate of TRL increments were observed relatively faster in Ψ_w -0.03, to marked in Ψ_w -0.15 MPa. TRL increased at a stable rate in Ψ_w -0.60 MPa from 13 to 25 d after transplanting. The RLWR (mm mg^{-1}) showed almost a linear reduction by decrease in Ψ_w from -0.03 to -1.10 MPa (Box in Fig. 6a). While averaging across Ψ_w, RLWR markedly increase with plant age from 7 to 19 d after transplanting with a non-significant change thereafter from 19 to 25 d after transplanting (Box in Fig. 6b). Moderate changes in Ψ_w (Table 1) or pot depth (Table 2) did not differ ($p \leq 0.05$) RLWR of wheat plant. Treatment Ψ_w with sampling interaction revealed higher RLWR in Ψ_w -0.03, followed by Ψ_w -0.15 and Ψ_w -0.60 MPa with lowest for Ψ_w -1.10 MPa in all samplings during 25 d growth after transplanting (Fig. 6d). RLWR in Ψ_w -0.03 MPa revealed marked (7 to 19 d) to stable increase (19 to 25 d) while in Ψ_w -0.15 MPa a more or less linear increase for 25 d after the transplanting. Contrary to that RLWR increased linearly for 7 to 19 d after transplanting but declined thereafter from 19 to 25 d after transplanting for Ψ_w -0.60 MPa. A very nominal increase observed in Ψ_w -1.10 MPa for 25 d growth after the transplanting in RLWR.

***Root volume and ratio*:** Root volume (ml) declined markedly in a linear fashion when Ψ_w decreases from -0.03 to -0.60 MPa but thereafter a moderate reduction was observed in the root volume with a further decreased in Ψ_w from -0.60 to -1.10 MPa (Fig. 7a). By average across Ψ_w,

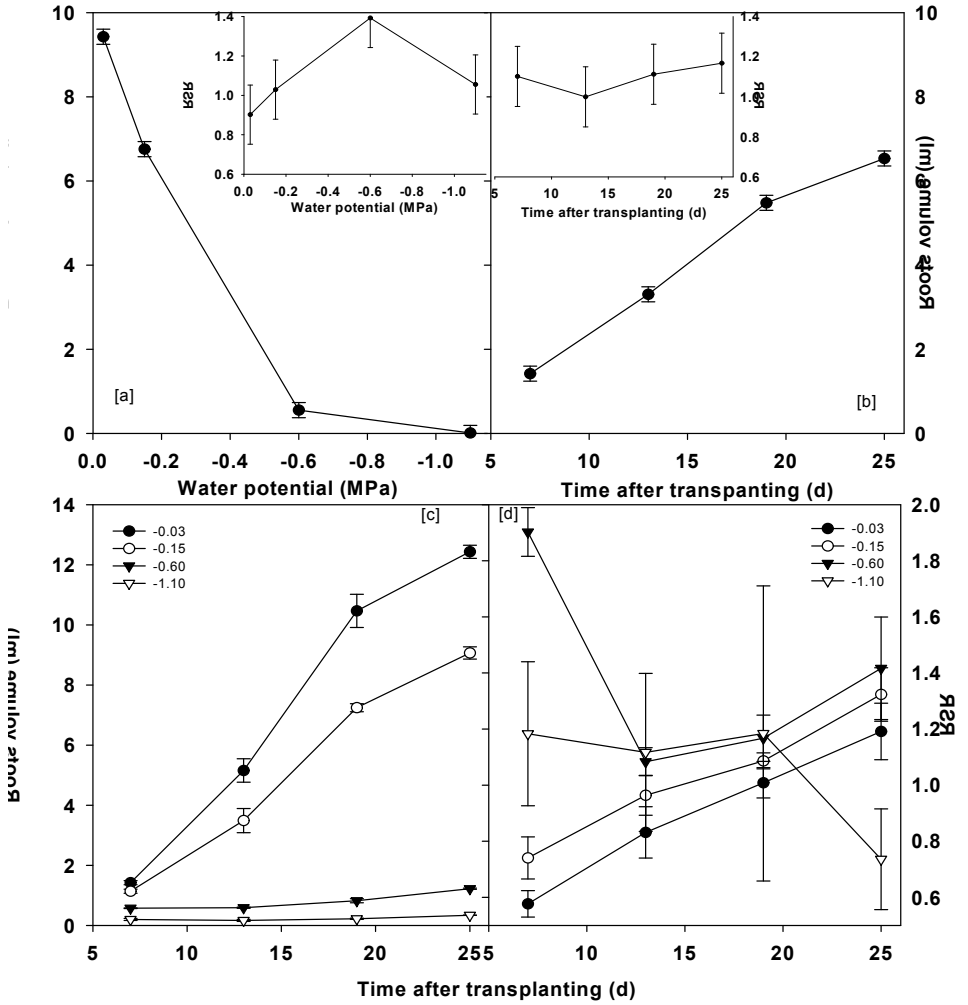

Figure 7. Different water potentials effects on roots volume (ml) of a young wheat plant [a] for 25 d after transplanting a germinated seed [b]. The interactions of Ψw and time after transplanting are also shown [c]. The inset figures show root roots to shoot ratio and the interactions are shown separately [d]. Letters (a, b & c) indicate statistically significance ($p \leq 0.001$) difference using HSD test.

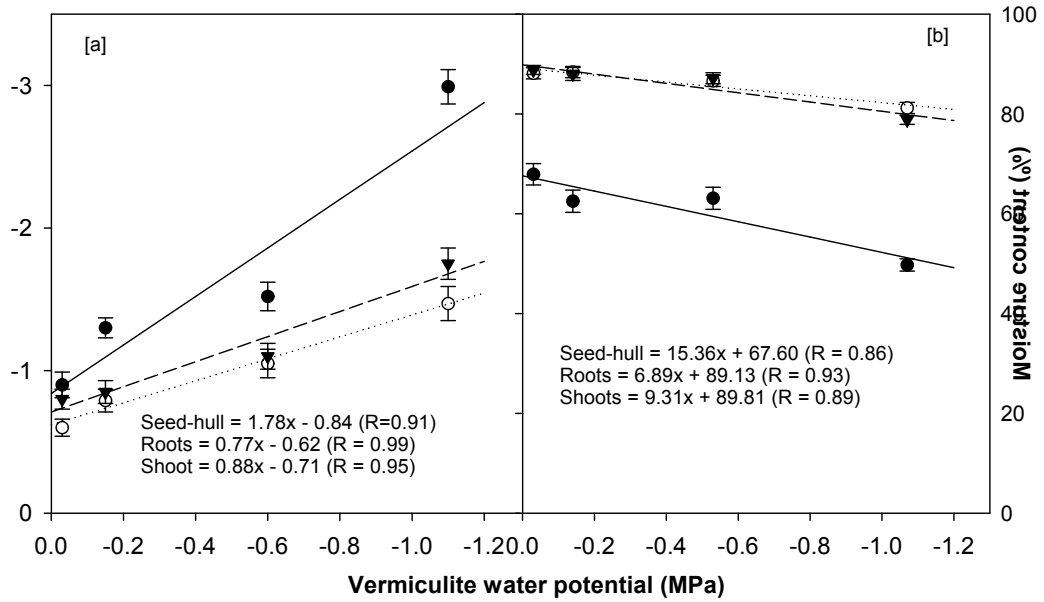

Figure 8. Different water potential effect on osmotic potential (MPa) and moisture contents (%) of a young wheat plant parts.

root volume showed a marked increment from 7 to 13 d after transplanting but with a moderate decrease between 19 and 25 d after transplanting (Fig. 7b). The roots volume (ml) did not change either with slight variations in Ψ_w from -0.03 and -0.11 or Ψ_w -0.07 and -0.15 MPa (Table 1) and/or limiting pot-depth from 30 to 10 cm (Table 2). Interaction of treatments Ψ_w x time after transplanting showed linear increase for Ψ_w -0.03 and -0.15 MPa from 7 and 19 d with a moderate reduction thereafter from 19 to 25 d after transplanting (Fig. 7c). Root volume of Ψ_w -0.60 and -1.10 MPa increased with a very slow and stable rate between 7 and 25 d after transplanting. Root volume increased very slowly in Ψ_w -1.10, relatively mild in Ψ_w -0.60, to high in Ψ_w -0.15 to the highest in Ψ_w -0.03 MPa for 25 d growth. The inset Fig. 8 shows root to shoot ratio (RSR). RSR increased when Ψ_w decreased from Ψ_w -0.03 to -0.06 MPa with a further reduction thereafter from Ψ_w -0.06 to -1.10 MPa (Box in Fig. 7a). RSR between 7 and 25 d after transplanting did not differ ($p \leq 0.05$) statistically (Box in Fig. 7b). Moderate reductions in Ψ_w (Table 1) or changing pot depth (Table 2) did not influence RSR. Interaction of Ψ_w and time after transplanting showed an increase in RSR for all Ψ_w treatments from 7 to 25 d after transplanting (Fig. 7d). RSR in Ψ_w -0.03 MPa was the lowest and linearly increased with time after transplanting, followed by a similar moderate increase with time for Ψ_w -0.15 MPa. Irrespective of day 7, RSR in Ψ_w -0.60 MPa was observed the highest between 13 and 26 d after transplanting. RSR in Ψ_w -1.10 remained almost stable from 7 to 13 d after transplanting with a sudden decline at 25 d after the transplanting.

***Parts osmotic potential and moisture content*:** Data showed that Ψ_o decreased by decreasing vermiculite Ψ_w in the plant parts, but with different rates (Fig. 8a). Among the plant parts, seed-hull showed the lowest Ψ_o, followed by shoot and the highest for roots at every Ψ_w treatment. Nonetheless, decreased in Ψ_o against reduction in treatment's vermiculite Ψ_w was observed in a linear fashion of a plant parts and can be estimate with a regression slope (*b*). The figures suggest slope values of 1.78 (r^2 =0.91) for seed-hull, 0.77 (r^2 = 0.99) for shoot and 0.71 (r^2 = 0.95) for roots against reductions in Ψ_w (MPa). A decrease in moisture contents (%) was also observed in plant parts for seven days growth after the transplanting from a high to low Ψ_w treatments (Fig. 8b). Figure showed a reduction in moisture contents with decreasing Ψ_w in all parts but with different rates in seed-hull, roots and shoot (Fig. 8a). The seed-hull showed low moisture contents than roots and shoots at all Ψ_w treatments with almost similar values for roots and shoots. The moisture contents decreased in a stable rate in different parts against decrease in the substrate Ψ_w that could be estimate from slopes of linear regression. We estimated slope values 15.36 (r^2 =0.86) for seed-hull, 6.89 (r^2 = 0.93) for roots and 9.31 (r^2 = 0.89) for shoot over a reduction in Ψ_w.

***Plant growth dynamics*:** Irrespective of vermiculite water potentials treatments, both shoot and roots dry matter showed exponential responses with the seed-hull dry matter (Fig. 9a). The relationships of both the shoot and roots with seed-hull were strongly positively correlated. Likewise, both the shoot and roots fractional dry matter showed a negative linear relationship with seed-hull fractional dry matter (Fig. 9b). As

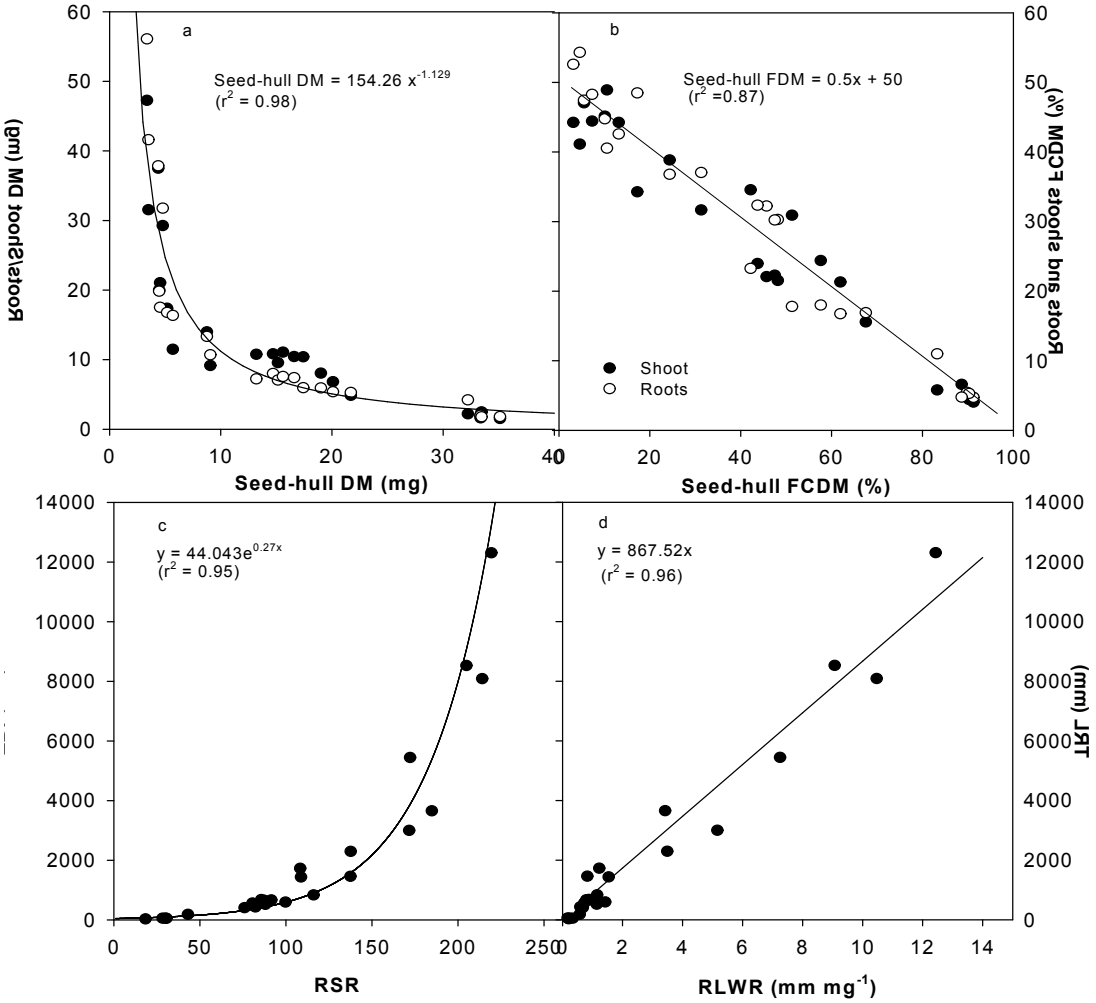

Figure 9. Relationship (a) seed-hull DM with roots- and shoot DM, (b) seed-hull FCDM with roots- and shoot FCDM, (c) roots-to-shoot ratio with total root length and (d) root length to weight ratio (RLWR) with total root length. Open symbols for roots and closed for shoot DM in window a and b.

substrate water potential decreased, the FCDM showed an increase in seed-hull and hence decrease both shoot and roots FCDM. While averaging across different water potential treatments, total root length of wheat plant showed an exponential growth with plant root to shoot ratio (Fig. 9c). Similarly, irrespective of different water potentials treatments, total plant roots length showed a linear relationship with root length to weight ratio (Fig. 9d).

DISCUSSION

We observed marked reductions in the leaf area, plant DM, plant roots and shoots DM, seminal roots length, branch length of seminal roots and roots volume of 25 d old wheat seedlings at different Ψ_w levels ranging from -0.03 to -1.10 MPa. It indicated that in 25 d of planting both roots and shoots would have shown similar responses in reduction of Ψ_w. The results indicated that shoot growth promoted ($p \leq 0.05$) with increasing moisture content for a germinated seed of wheat that is known as drought tolerant (Saidi *et al*., 2010). Reduction in Ψ_w for seedling has already been shown mild to marked ($p \leq 0.05$) decreases in the leaf area, tiller number, spike length, grain index, seedling's height and yield (Kramer and Boyer, 1995; Bayoumi *et al*., 2008). Reduction in the soil Ψ_w has already shown a decrease ($p \leq 0.05$) in germination, coleoptile length, shoot and roots' length, fresh shoot and roots masses in previous experiments (Chandler and Singh, 2008; Khakwani *et al*., 2011). Plant growing trait has also shown marked sensitivity to changes in soil moisture (Chandler and Singh, 2008). We know that growth is results of cell divisions and cell enlargements and regulations of the cell extension are critical for crop growth and morphology (Smith, 2003). Stress has confined growth by retarding cell division and -extension, especially under

low Ψ_w (Gao *et al*., 2007) to understand the physiological mechanisms of crop adaptation to low Ψ_w (Riera *et al*., 2005). The findings of present study clearly indicated that both root and shoot growth was promoted at higher Ψ_w in wheat seedling, which is known as a relatively drought-resistant crop when compared with other cereals e.g. maize. As reported earlier, leaf area decreased ($p \leq 0.05$) markedly with reduction in soil water potential (Kramer and Boyer, 1995; Lambers *et al*., 1998). It is highly sensitive to drought. Nonetheless, growth responses of roots to a reduction in Ψ_w were observed relatively mild than the shoot (Fig. 9). We observed almost similar responses for wheat roots and shoot growth for 25 d by reduction in the substrate Ψ_w. Literature also confirmed that healthy seeds produced healthy crop stand even in an un-favorable environment (Haque *et al.*, 2007; Kalakanavar *et al*., 1989; Hampton, 1981). It is, therefore, important to understand the responses of Ψ_w for germination and thereafter seedling conversion to a healthy plant (Saidi *et al*., 2010; Mian and Nafziger, 1992) for optimum productivity.

Mostly in arid and semiarid regions, wheat faced early drought stress that mainly induced emergence and/or mots probably the early seedling growth (Bouaziz and Hicks, 1990). Water shortage at this stage of the crop growth has a serious concern for germination and thereafter the crop stand establishment if associated together with low temperature of the following phases of crop development. No doubt germination markedly affected adversely by dropout in soil but the critical lower limit of germination for the external water potential vary among genotypes (Pratap and Sharma, 2010). Both seed size and post emergence environmental conditions may interact with moisture and/or Ψ_w for wheat seedling early growth. Our findings of increased in roots and shoot DM by increasing Ψ_w agree with published literature (Gazal *et al.*, 2004; Saidi *et al.*, 2010). However, the lowest treatment (e.g. Ψ_w -1.10 MPa) was unable to produce any shoot and/or roots DM, which shows that a germinated seed of wheat crop in Ψ_w -1.10 MPa is unable to sustain growth if faced by drought right at germination. Likewise, FCLA for leaf 1 and 2 is interesting estimation of seedling establishment and has showed a similar reduction ($p \leq 0.05$) by decrease in substrate Ψ_w. As reported earlier, leaf growth is sensitive to losses or changes in substrate Ψ_w. It is the initial growth process that affected by decrease in leaf water potential (Bargali and Tewari, 2004). FCLA showed a significant ($p \leq 0.05$) decrease by reduction in Ψ_w suggesting that fractional leaf loss is determined primarily by limiting water availability to the seedling early growth (Farooq *et al*., 2009). Early roots and shoot growth of a young plant are primarily depends on progressive expansion in the plant leaf area in the early phases of development (Mahdid *et al*., 2011).

Plant DM was high (Ψ_w -0.03 and -01.5 MPa) to evidently low at low substrate moisture contents (Ψ_w -0.60 and -1.10 MPa). We noticed an increase ($p \leq 0.05$) in wheat plant DM at Ψ_w -1.10 MPa due to increase in seed-hull DM. Both shoot and roots DM showed consistent decreases. The literature has also confirmed a consistent reduction in a young plant DM by decreasing moisture contents of the substrate, which mainly associate with marked reduction ($p \leq 0.05$) in shoot DM (Hirasawa *et al*., 2005; Martin *et al*., 2006) and sporadically in the roots with shoot (Hirasawa *et al*., 1994; Akmal and Hirasawa, 2004). Seed hull is primary initiator of plan DM, but generally ignored while measuring DM in early stage of a seedling growth. As expected, dry matter of plant increased with time by expansion in roots and shoot DM (Saidi *et al*., 2010). However, the seed-hull DM showed an increase by decrease in vermiculite Ψ_w confirming that low moisture at Ψ_w -0.60 MPa was not successful transferring all food reserve to the growing plant. Shortage of moisture in substrate at this phase of the crop growth adversely affected seedling establishment, which might be unable to compete in growth to attain the optimum size or volume. Irrespective of the Ψ_w treatments, FCDM of roots and shoot showed a linear decreasing trend against the FCDM of seed-hull (Fig. 9) confirming significance of the seed-hull DM under different Ψ_w treatments. Space availability or depth limitation do not influenced seminal root length and/or branch number but reduction in vermiculite Ψ_w did that affecting the shoot growth and primarily with marked differences in leaf area and FCLA for each developing leaf of the young plant (Boonjung and Fukai, 1996; Sahnoune *et al*., 2004; Akmal and Hirasawa, 2004). Some researchers have also observed an increase in the seminal roots length under mild reduction of substrate Ψ_w due to stimulating cell expansion and elongation (Sharp *et al*., 2004) but we noted a significant ($p \leq 0.0\%$) decreases both in root length and branch number that has affected roots volume accordingly when vermiculite moisture decreased (Himmelbauer *et al*., 2004; Waines and Ehdaie, 2007). It is quite natural that root elongation in length, branch number expands with initiation, emergence, and growth of lateral roots from the root pericycle and epidermis. Root branch comprises a significant proportion of the root system (Waines and Ehdaie, 2007; Shah *et al*., 2012) which if allow to expand optimum under sufficient moisture condition of the substrate (Yoshida *et al*., 1982), would might have resulted healthy plants right after emergence that would ensure the optimum production. We know that the highest mass or length of a plant roots ensures seedling resistant to stay longer under drought stress conditions if or when temperature is low and seedling is exposed to cope with the unfavorable environments of their surroundings.

The study suggests that decreasing Ψ_w of the substrate has shown marked ($p \leq 0.05$) changes in roots and shoot growth, which adversely affected the seedling establishment in the

early development phases of its establishment. The substrate moisture is a fundamental component of transferring assimilates from seed reserve to the growing seedling that is responsible for a healthy crop stand and optimum productivity. Wheat being relatively drought resistant has shown marked differences ($p \leq 0.05$) for both roots as well as shoot growth dynamics by decreasing substrate moisture content in the early phase of plant establishment. However, decrease in pot's depth did not influence rooting growth, establishment and dry matter as influenced by the shortage of substrate moisture contents.

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