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In vitro screening of alfalfa (*Medicago* spp.) genotypes root traits and PEG-6000 induced drought stress

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Abstract
Drought severely limits global plant distribution and agricultural production. Elucidating the physiological mechanisms governing alfalfa stress responses will contribute to the improvement of drought tolerance in leguminous crops. To understand the adaptation mechanism of alfalfa (*Medicago* spp.) to drought stress, growth, and physiological parameters by using PEG-6000 and agar gel were measured under simulated levels (0%, 5%, 10% and 20%) of PEG and agar gel (1-2%). Fourty-five genotypes of alfalfa were evaluated comprising of genotypes from Mongolia, Italy, Cortia and Ladakh. Results indicated significant differences among the genotypes. In PEG-6000 mediated experiment with increase in concentration of PEG, the significant decrease in primary root length (cm), number of laterals and root weight (g) was observed. Based on results, the genotypes AUS-ALF-12, AUS-ALF-17exhibited lesser reduction in primary root length, and genotypes AUS-ALF-20, AUS-ALF-18 exhibited lesser reduction in number of laterals and genotypes AUS-ALF-4, ALF-IT-7 exhibited lesser reduction in root weight. while as, in agar based experiment the measurement of traits *viz.*, primary root length, number of lateral roots, root weight and total root length were observed and based on results the genotypes ALF-CRO-4, AUS-ALF-15 exhibited lesser reduction in primary root length and genotypes ALF-CRO-8, ALF-Kar-10 exhibited lesser reduction in number of laterals, genotypes ALF-CRO-8, ALF-Kar-9 exhibited lesser reduction in root weight, genotypes ALF-CRO-8, ALF-CRO-9 exhibited lesser reduction in total root length. Hence, progressive decline was observed across all genotypes with an increase in PEG concentration, and also in agar experiment, respectively.

Keywords: PEG, agar, root length, root weight

Introduction
Drought is a significant abiotic stress that severely restricts the geographic range and productivity of crop plants globally, resulting in significant yield losses (Farooq *et al*., 2009; Fang and Xiong, 2015; Cao *et al*., 2017) [6, 4, 2]. Around one-third of the world's land area, according to Sivakumar *et al*. (2005) [2], is made up of dry and semi-arid regions. Global warming, deforestation and urbanisation will contribute to more frequent and severe droughts in many regions in the future. Because of this, there is a higher need to breed new crops with superior drought resistance and higher yields when there is a water shortage (Fulda *et al*., 2011; Joshi *et al*., 2016) [7, 12]. Understanding the physiological mechanisms of drought stress tolerance at different plant developmental stages is necessary for the creation of crops with increased drought tolerance (Farooq *et al*., 2009, 2016) [6]. One of the most extensively grown feed crops, alfalfa (*Medicago sativa* L.) has great agricultural qualities and a high commercial value. In dry and semi-dry environments, the deep roots of alfalfa help prevent soil erosion (Quan *et al*., 2015) [19]. Compared to other food crops, alfalfa has a comparatively high level of drought tolerance (Kang *et al*., 2011; Tang *et al*., 2014) [13, 23]. The roots of a plant are vital parts that supply it with nutrients and water. The modification of a plant's root development and the concomitant stress response mechanisms are significantly correlated with the plant's sensitivity or tolerance to unfavourable environmental conditions (Sengupta *et al*., 2011) [21].
PEG is a commonly used osmotic agent that causes osmotic stress in plants by lowering the water potential of nutrient solutions, which makes less water available to the plant roots. PEG is non-absorbable, non-metabolized and non-toxic (Joshi *et al*., 2011) [11]. PEG solutions have been conceptually well established as being better suitable for simulating water deprivation in plants and evaluating their capacity to withstand drought at the seedling stage. (Hadi *et al*., 2014) [8].

Inspite of roots playing important role of absorption of water and nutrients, they also provide anchorage to plants. The contents of rhizophere (immediate vicinity of root) highly varies in space and time. Measurement of root growth and architecture is therefore necessary to understand complex interactions of roots and understanding the adaptability of plants. Roots architecture can be studied in the agar gel plates (1-2%). so, the objective of present study was to examine the *in vitro* drought tolerance of alfalfa genotypes in PEG and Agar solutions, respectively.

Experimental details

An experiment was carried out during 2021, containing 45-genotypes of Alfalfa (*Medicago sativa*, *Medicago falcata* and *Medicago varia*) comprising of genotypes from Mongolia, Italy, Cortia and Ladakh and were evaluated for *invitro* drought tolerance. The design followed was completely randomized design (CRD) with three replications.

Root traits analysis using agar solution

The effect of water stress was induced by using the agar solution**.** For the measurement of traits *viz.*, primary root length, number of lateral roots, root weight and total root length, seeds were germinated in transparent gel (2% agar) filled in plastic petri plates (Christopher *et al*., 2012) [3]. Four seeds for each genotype was surface sterilized with 0.5 per cent NaOCl for one minute, rinsed thoroughly with distilled water and was put in the petri plates containing moist filter paper. Two days after, the seeds germinated and the radicle emerged and were transferred to the 15x15 cm^2 plastic petri plates containing 2 per cent sterilized solid agar medium (2% w/v) in darkness in germinator at 25 ^oC. The germinating seeds were placed from cut sides of the petri plates with radicle inserted into the agar and kept for six days under darkness at room temperature and after six days data were recorded for primary root length, number of lateral roots, root length, root biomass, respectively.

***In-vitro* water stress using PEG-6000**

The effect of water stress was induced by different osmotic potential levels (Control (0%), 5, 10 and 20 per cent of PEG-6000 treatments on germination. Four seeds from each genotype of alfalfa were surface sterilized with 0.5 per cent NaOCl for one minute, rinsed thoroughly with distilled water and were put in petri plates containing moist filter paper with different concentrations of PEG and allowed to germinate in a germinator at 25 ^oC and 75 per cent humidity in darkness. Primary root length (cm), number of laterals, root weight was measured after seven days.

Results and Discussion

Agar culture experiment

The genotypes used in the present study were evaluated for various traits recorded in agar experiment as given in Table-1. The mean value for primary root length (cm) had a mean value of 16.532 with highest value recorded in ALF-CRO-4 (29.033) followed by AUS-ALF-15 (28.067) followed by ALF-CRO-9 (26.000) followed by ALF-CRO-8 (25.000) and lowest was recorded in ALF-IT-3 (7.000) followed ALF-Kar-10 (11.400) followed by ALF-kar-9 (12.333) and AUS-ALF-8 (12.400). while as, the number of laterals had a mean value of 8.561 with highest value recorded in ALF-CRO-8 (20.000) followed by ALF-Kar-10 (15.000) followed by ALF-Kar-9 (15.000) followed by ALF-CRO-1 (13.333) and lowest was recorded in AUS-ALF-14 (0.004) followed by AUS-ALF-23 (0.004) followed by AUS-ALF-8 (0.004) and AUS-ALF-6 (0.004). while as, the root weight (g) had a mean value of 0.011 with highest value recorded in ALF-CRO-8 (0.020) followed by ALF-Kar-9 (0.019) followed by AUS-ALF-20 (0.017) followed by ALF-CRO-9 (0.016) and lowest was recorded in AUS-ALF-14, AUS-ALF-23, AUS-ALF-8 and AUS-ALF-6 (0.004).while as, the total root length (cm) had a mean value of 27.856 with highest value recorded in ALF-CRO-8 (54.300) followed by ALF-CRO-9 (40.733) followed by ALF-IT-4 (35.800) followed by ALF-CRO-3 (35.767) and lowest was recorded for ALF-IT-3 (13.667) followed by ALF-Kar-10 (18.433) followed by ALF-Kar-9 (19.267) and ALF-CRO-5 (21.000).

The spatial and temporal structure of roots in the growth medium is known as root system architecture (RSA), and this considerably affects a plant's capacity to capture water and nutrients. RSA reduces the capacity of several plant activities in alfalfa, including symbiotic nitrogen fixation, nutrient absorption, and water use efficiency (Salter *et al*. 1994). In a study on the inheritance of root morphological features, it was discovered that the environment had the least influence on the number of lateral roots, the position of lateral roots, and the number of fibrous roots (Johnson *et al.* 1996) [10]. There are several benefits to breeding for certain root properties for enhancing alfalfa cultivars. By focusing on specific root features, alfalfa harvest and stand longevity may be improved. The alfalfa root system, in particular the taproot, serves as a carbon storage organ to supply regrowth following herbage harvest and for regrowth in the spring. The roots' in determinant meristems contribute to the perennial nature of alfalfa (Munné-Bosch, 2014) [18], however, It has not been investigated how root characteristics like taproot diameter or root dry matter affect alfalfa's ability to survive and persist during the winter. A deep tap root also lessens competition from shallow rooted plants like fodder grasses that may be inter planted with alfalfa and enhances possible access to water resources to promote drought tolerance (Voss-Fels *et al.* 2018) [24].

***In vitro* response of alfalfa (*Medicago sativa* L.) genotypes to PEG stress**

The genotypes used in the present study were evaluated for response to PEG 6000 given in Table-2 at different levels (0, 5, 10 and 20%) and the results were recorded. The mean value of primary root length under 0% level of PEG-6000 had a mean value of 7.576 with highest value recorded for AUS-ALF-20 (12.000) followed by AUS-ALF-10 (11.000) and lowest was recorded for ALF-IT-4 (4.200) followed by ALF-CRO-5 (4.300). At 5% level of PEG- 6000, the primary root length had a mean value of 6.884 with highest value recorded for AUS-ALF-20 (11.300) followed by AUS-ALF-11 (9.700) and lowest was recorded for ALF-CRO-10 and ALF-IT-4 (3.200). At 10%-level of PEG-6000, the primary root length had a mean value of 5.884 with highest value recorded for AUS-ALF-11 (10.000) followed by AUS-ALF-13 (8.8000 and lowest was recorded for ALF-IT-4 (1.300) followed by ALF-CRO-5 (2.000). At 20%-level of PEG-6000, the primary root length had a mean value of 4.522 with highest value recorded for AUS-ALF-12 (8.500) followed by AUS-ALF-17 (8.000) and lowest was recorded for ALF-IT-4 (1.300) followed by ALF-IT-3 (1.100), respectively. While as, the mean value of number of laterals under 0%-level of PEG-6000 had a mean value of 4.978 with highest value recorded for AUS-ALF-6 and AUS-ALF-18 (8.000) and lowest was recorded for AUS-ALF-14 (2.000) followed by AUS-ALF-22 (3.000). At 5%-level of PEG-6000, had a mean value of 3.222 with highest value recorded for AUS-ALF-6 and AUS-ALF-18 (6.000) and lowest was recorded for AUS-ALF-14 and AUS-ALF-16 (1.000). At 10%-level of PEG-6000, the number of laterals had a mean value of 2.015 with highest value recorded for AUS-ALF-18 and AUS-ALF-20 (4.000) and lowest was recorded for AUS-ALF-14 (0.000) followed by AUS-ALF-23 (0.667). At 20%-level of PEG-6000, the number of laterals had a mean value of 1.104 with highest value recorded for AUS-ALF- 20 and AUS-ALF-18 (3.000) and lowest was recorded for AUS-ALF-14 and AUS-ALF-17 (0.000), respectively. while as, the mean value for root weight at 0%-level of PEG- 6000, had a mean value of 0.041with highest value recorded for AUS-ALF-4 (0.140) followed by ALF-IT-8 (0.092) and lowest was recorded for ALF-CRO-5 (0.010) followed by ALF-CRO-4 (0.011). At 5% level of PEG-6000, the root weight had a mean value of 0.035 with highest value recorded for AUS-ALF-4 (0.090) followed by ALF-Kar-9 (0.084) and lowest was recorded for ALF-CRO- 5 (0.003) followed by ALF-CRO-4 (0.007). At 10%-level of PEG-6000, the root weight had a mean value of 0.030 with highest value recorded for AUS-ALF-4 (0.120) followed by ALF-IT-7 (0.080) and lowest was recorded for ALF-CRO-5 (0.002) followed by ALF-CRO-4 (0.003). At 20%-level of PEG-6000, the total root weight had a mean value of 0.023 with highest value recorded for AUS-ALF-4 (0.110) followed by ALF-IT-7 (0.076) and lowest was recorded for ALF-CRO-5 and ALF-CRO-4 (0.001), respectively.

Drought stress severely reduces germination rate and seedling establishment, growth and survival, which are critical factors in determining plant productivity (Kashif 2011) [14]. Our results were in agreement with the study of Farooq *et al*. 2009 [6]. Osmotic pressure of the soil solution rises with increasing levels of drought stress, which frequently causes water scarcity, cell dehydration, plant wilting and withering, and occasionally death (Farooq *et al.* 2009) [6]. PEG blocks the water flow routes, limiting water absorption and leading to desiccation of the plant, similar to actual drought conditions (Lawlor, 2010) [15]. As a result, PEG is widely utilised in experiments to artificially incite drought stress. (Boldaji *et al.* 2012; Jatoi *et al*. 2014; Mouradi *et al.* 2016) [1, 9, 17]. However, the amount of oxygen delivered as a result of the difference in growth media may be the main distinction between PEG-induced and true drought stress. The diffusion of oxygen gas can satisfy the oxygen requirements for root growth and metabolism during actual drought stress when soil is used as the growth medium. (Xing *et al.* 2005) [25]; because of the restricted oxygen solubility in the nutrient solution, the root system may not receive enough oxygen during PEG-induced drought stress when nutrient solution is being employed as a growth medium. This difference in oxygen availability may potentially affect the growth and development of plants under PEG-induced drought stress. Because crops with deeper roots have better access to stored water and nutrients like nitrogen, a soluble nutrient that tends to seep into the deeper layers of the soil, rooting depth is one of the most often studied traits. Water and nutrients are heterogeneously distributed in soil so that the distribution of roots (their architecture) will markedly affect the ability of a plant to secure these soil-based resources (Lynch, 1995) [16].

Analysis of variance for root traits scored under laboratory screening

Analysis of variance for various root traits scored under laboratory conditions in both the experiments is presented in Table-3 and Table-4 which shows that mean square due to genotypes was significant for all the traits.

Conclusion

Keeping in view the above stated research finding, it can be concluded that the genotypes exhibited reduction in all the parameters with increase in level of PEG-6000 and also restriction of root growth occurs across the genotypes in agar solutions. So, based on seedling phenotyping, developmental differences between branch-rooted and tap-rooted plants were identified in this study. A practical method to change root system architecture (RSA) at plant maturity and speed up the breeding cycles leading to specific root phenotypes is to select for divergent root characteristics in alfalfa seedlings. Future selections for these root traits would further streamline the breeding process.

Table 1: Mean performance of Alfalfa (*Medicago sativa* L.) genotypes for root traits under laboratory in Agar culture experiment

Genotype	Primary root length (cm)	No. of laterals	Root weight (g)	Total root length (cm)
ALF-CRO-1	21.167	13.333	0.014	35.367
ALF-CRO-2	18.000	10.667	0.016	34.233
ALF-CRO-3	21.333	12.667	0.012	35.767
ALF-CRO-4	29.033	6.000	0.005	21.333
ALF-CRO-5	13.800	10.000	0.013	21.000
ALF-CRO-6	13.533	11.000	0.012	27.133
ALF-CRO-7	14.000	8.333	0.015	32.667
ALF-CRO-8	25.000	20.000	0.020	54.300
ALF-CRO-9	26.000	5.333	0.016	40.733
ALF-CRO-10	18.667	12.267	0.014	24.833
ALF-CRO-11	16.067	10.667	0.013	27.033
ALF-CRO-12	16.233	6.333	0.006	25.333
ALF-IT-1	16.100	8.000	0.014	28.300
ALF-IT-2	14.800	7.000	0.013	25.133
ALF-IT-3	7.000	12.000	0.006	13.667
ALF-IT-4	22.333	12.333	0.014	35.800
ALF-IT-5	22.433	6.667	0.006	30.667
ALF-IT-6	15.033	7.000	0.016	29.100

ALF-IT-7	14.233	8.333	0.011	22.467
ALF-IT-8	17.000	8.000	0.013	31.033
ALF-Kar-9	12.333	15.000	0.019	19.267
ALF-Kar-10	11.400	15.000	0.016	18.433
AUS-ALF-1	12.867	6.667	0.015	28.633
AUS-ALF-2	14.300	6.667	0.016	28.300
AUS-ALF-3	15.100	8.667	0.007	32.467
AUS-ALF-4	17.933	7.000	0.013	32.433
AUS -ALF-5	16.200	7.000	0.015	31.033
AUS-ALF-6	15.133	5.667	0.004	21.300
AUS-ALF-7	12.633	7.000	0.006	22.533
AUS-ALF-8	12.400	5.000	0.004	23.633
AUS-ALF-9	13.033	7.000	0.008	28.400
AUS-ALF-10	13.633	8.000	0.006	21.367
AUS-ALF-11	13.000	8.000	0.006	21.267
AUS-ALF-12	23.900	9.000	0.015	28.467
AUS-ALF-13	12.400	8.000	0.007	23.433
AUS-ALF-14	16.200	7.000	0.004	32.200
AUS-ALF-15	28.067	6.333	0.015	31.667
AUS-ALF-16	15.700	7.333	0.014	23.567
AUS-ALF-17	14.800	7.667	0.014	24.900
AUS-ALF-18	14.433	6.000	0.014	24.767
AUS-ALF-19	18.800	6.000	0.012	27.100
AUS-ALF-20	13.933	8.000	0.017	26.300
AUS-ALF-21	14.467	6.000	0.006	29.900
AUS-ALF-22	13.267	6.000	0.007	29.033
AUS-ALF-23	16.233	5.333	0.004	27.233
Mean	16.532	8.561	0.011	27.856
CD ($p \leq 0.05$)	3.028	2.451	0.004	2.451
CV	11.273	17.621	22.059	5.415

Table 2: Mean response of Alfalfa (*Medicago sativa* L.) genotypes to PEG mediated screening

Genotype	Primary root length (cm)				No. of laterals				Root weight (g)			
	0%	5%	10%	20%	0%	5%	10%	20%	0%	5%	10%	20%
ALF-CRO-1	6.400	5.867	5.600	4.200	7.000	4.000	2.000	1.000	0.029	0.017	0.013	0.010
ALF-CRO-2	7.300	7.200	3.367	1.800	5.000	3.000	3.000	1.000	0.015	0.013	0.009	0.005
ALF-CRO-3	5.600	5.400	4.900	2.300	6.000	4.000	3.000	2.000	0.016	0.013	0.012	0.007
ALF-CRO-4	6.100	5.800	3.600	1.400	3.000	3.000	1.000	0.000	0.011	0.007	0.003	0.001
ALF-CRO-5	4.300	4.000	2.000	1.900	4.000	3.000	2.000	1.000	0.010	0.003	0.002	0.001
ALF-CRO-6	6.500	5.800	5.100	4.500	5.000	3.000	2.000	1.000	0.023	0.021	0.017	0.013
ALF-CRO-7	8.100	6.800	6.300	5.000	6.000	5.000	3.000	2.000	0.020	0.014	0.010	0.009
ALF-CRO-8	8.700	5.900	4.200	3.300	5.000	4.000	2.000	1.000	0.032	0.035	0.031	0.027
ALF-CRO-9	6.300	6.000	5.900	5.700	4.000	3.000	1.000	1.000	0.011	0.014	0.013	0.012
ALF-CRO-10	4.500	3.200	2.100	1.200	6.000	5.000	4.000	2.000	0.016	0.015	0.012	0.011
ALF-CRO-11	6.700	6.300	6.000	5.300	5.000	3.000	2.000	1.000	0.029	0.022	0.021	0.017
ALF-CRO-12	5.100	4.900	3.200	2.100	4.000	2.000	1.000	0.000	0.032	0.036	0.030	0.028
ALF-IT-1	5.200	4.100	3.800	3.200	6.000	5.000	3.000	2.000	0.012	0.010	0.007	0.005
ALF-IT-2	5.900	5.700	5.300	2.100	5.000	4.000	2.000	1.000	0.040	0.030	0.020	0.007
ALF-IT-3	6.100	5.800	4.200	1.100	4.000	2.000	1.000	0.000	0.014	0.010	0.008	0.004
ALF-IT-4	4.200	3.200	1.300	1.000	5.000	3.000	2.000	1.000	0.023	0.011	0.009	0.007
ALF-IT-5	6.400	6.100	5.800	3.900	7.000	4.000	2.000	1.000	0.037	0.028	0.020	0.011
ALF-IT-6	7.600	7.100	6.900	6.300	5.000	4.000	3.000	1.000	0.076	0.068	0.066	0.051
ALF-IT-7	8.100	7.600	7.500	6.100	6.000	3.000	2.000	1.000	0.087	0.082	0.080	0.076
ALF-IT-8	9.400	8.800	7.700	5.200	4.000	2.000	2.000	0.000	0.092	0.071	0.068	0.051
ALF-Kar-9	9.300	8.300	7.200	4.100	6.000	5.000	4.000	1.000	0.087	0.084	0.071	0.068
ALF-Kar-10	8.300	7.600	6.400	4.200	5.000	4.000	3.000	1.000	0.067	0.062	0.053	0.041
AUS-ALF-1	7.200	7.000	6.200	4.700	6.000	5.000	3.000	2.000	0.033	0.032	0.028	0.022
AUS-ALF-2	6.800	6.600	5.300	5.100	5.000	4.000	2.000	1.000	0.036	0.031	0.022	0.015
AUS-ALF-3	7.700	7.100	6.800	6.400	5.000	3.000	1.000	1.000	0.022	0.031	0.020	0.019
AUS-ALF-4	6.300	6.500	5.500	1.300	6.000	4.000	3.000	2.000	0.140	0.090	0.120	0.110
AUS-ALF-5	8.600	7.200	5.600	4.200	4.000	2.000	1.000	1.000	0.023	0.016	0.014	0.007
AUS-ALF-6	10.500	9.300	6.000	5.200	8.000	6.000	4.000	2.000	0.041	0.040	0.035	0.029
AUS-ALF-7	9.100	8.800	8.500	7.200	4.000	1.000	1.000	0.000	0.055	0.053	0.051	0.047
AUS-ALF-8	7.300	7.100	6.800	6.100	3.000	2.000	1.000	1.000	0.037	0.035	0.022	0.012
AUS-ALF-9	10.200	9.300	8.800	7.200	4.000	2.000	2.000	1.000	0.042	0.039	0.033	0.036
AUS-ALF-10	11.000	6.000	5.700	3.200	5.000	3.000	1.000	1.000	0.042	0.031	0.020	0.010

AUS-ALF-11	10.600	9.700	10.000	7.000	4.000	2.000	2.000	1.000	0.037	0.016	0.020	0.010
AUS-ALF-12	9.000	8.000	6.500	8.500	4.000	1.000	1.000	0.000	0.023	0.021	0.025	0.021
AUS-ALF-13	9.600	9.300	8.800	7.100	6.000	4.000	2.000	1.000	0.054	0.044	0.032	0.027
AUS-ALF-14	7.200	6.900	6.500	5.200	2.000	1.000	0.000	0.000	0.066	0.057	0.044	0.031
AUS-ALF-15	8.000	6.900	6.500	5.200	6.000	4.000	2.000	2.000	0.032	0.030	0.021	0.013
AUS-ALF-16	7.700	7.500	6.200	6.100	4.000	1.000	1.000	1.000	0.077	0.073	0.068	0.013
AUS-ALF-17	8.200	8.000	8.100	8.000	3.000	2.000	1.000	0.000	0.051	0.049	0.047	0.043
AUS-ALF-18	6.300	5.700	5.400	5.100	8.000	6.000	4.000	3.000	0.056	0.048	0.046	0.022
AUS-ALF-19	5.200	4.800	4.300	3.200	7.000	4.000	2.000	2.000	0.044	0.041	0.037	0.021
AUS-ALF-20	12.000	11.300	8.200	4.500	6.000	5.000	4.000	3.000	0.040	0.029	0.017	0.015
AUS-ALF-21	10.100	9.500	6.400	5.200	4.000	2.000	1.000	1.000	0.051	0.049	0.038	0.026
AUS-ALF-22	9.400	9.100	8.400	6.200	3.000	1.000	1.000	1.000	0.041	0.039	0.019	0.011
AUS-ALF-23	6.800	6.700	5.900	5.700	4.000	2.000	0.667	0.667	0.018	0.015	0.011	0.007
Mean	7.576	6.884	5.884	4.522	4.978	3.222	2.015	1.104	0.041	0.035	0.030	0.023
C.D ($p \leq 0.05$)	Genotype = 0.080				Genotype = 0.800				Genotype = 0.001			
	PEG Levels = 0.024				PEG Levels = 0.239				PEG Levels = 0.000			

Table 3: Analysis of variance for root traits under PEG-6000 mediated screening

Source of variation	Df	Primary root length (cm)	No. of laterals	Root weight (g)
Genotype	44	34.638**	12.833**	0.006**
Replication	3	237.308	378.514	0.008
Genotype x replication	132	2.489	1.021	0.000
Error	360	0.010	0.993	0.000

**Significant at 0.05% level.

Table 4: Analysis of variance for root traits scored under laboratory screening in alfalfa (*Medicago sativa* L.) genotypes

Source of variation	Df	Primary root length	No. of laterals	Root weight	Total root length
Genotypes	44	63.799**	29.115**	0.003**	133.936**
Error	90	3.473	2.276	0.001	2.276

**Significant at 0.05% level.

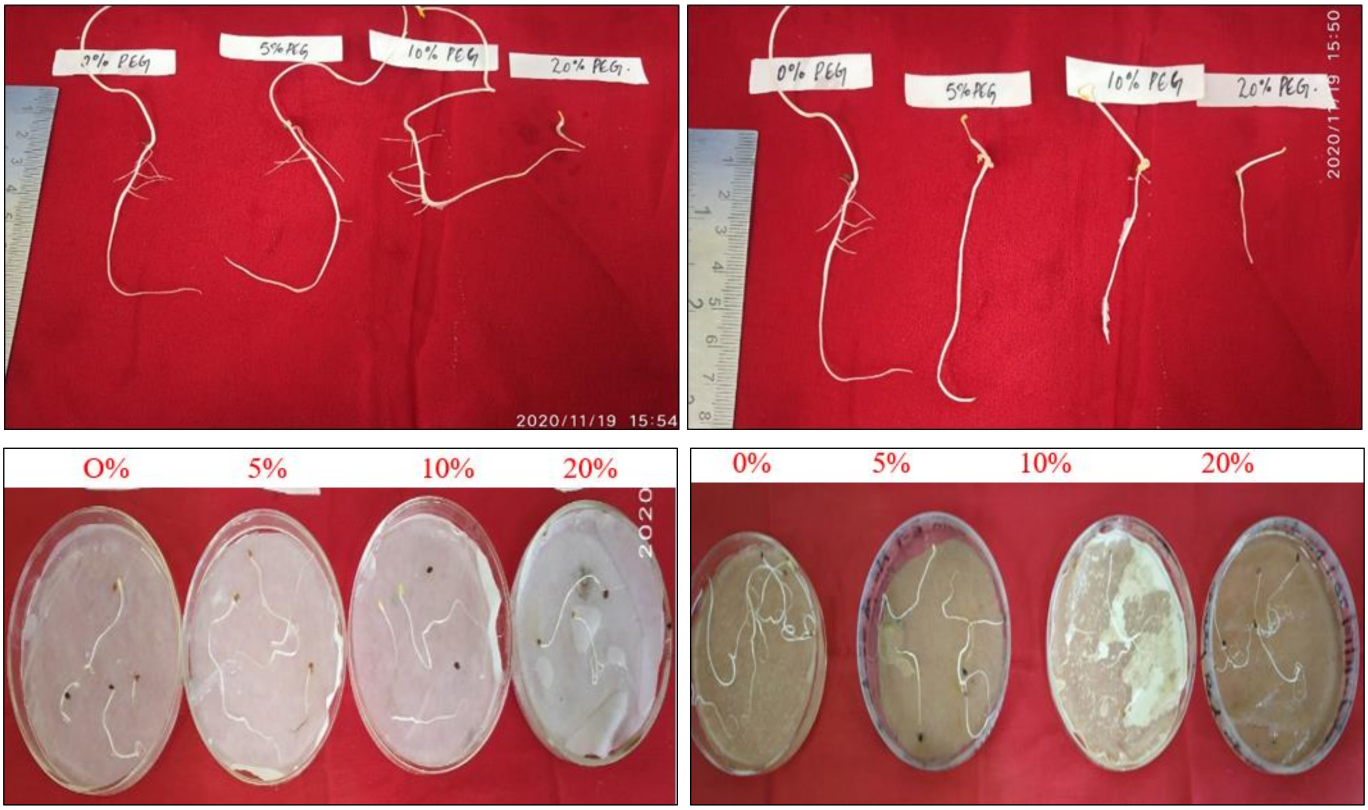

Fig 1: PEG-6000 mediated screening of Alfalfa (*Medicago* spp.)

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