

PHYSICOCHEMICAL RESPONSE OF ALFALFA (*MEDICAGO SATIVA* L.) AND RYE GRASS (*LOLIUM PERENNE* L.) GENOTYPES TO INDUCE DROUGHT STRESS AT SEEDLING STAGE

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Abstract. Alfalfa (*Medicago sativa* L.) is an important forage crop that is often grown in areas that frequently experience drought and water shortage. It is predicted that climate changes may increase the risk of local droughts, with severe consequences for agricultural practices. Here, we are illuminating the physicochemical mechanisms governing alfalfa and ryegrass (*Lolium perenne* L.) stress responses, contribute to the improvement of drought tolerance in leguminous crops. This study evaluated the performance of different alfalfa and rye grass genotypes against polyethylene glycol (PEG) induced drought stress. Among different, traits germination percentage, survival percentage, root and shoot length, fresh and dry root weights, fresh and dry shoot weights, total fresh weight of seedling, vigor index and proline content were found highly significant. On the basis of mean performance, the genotypes named Haymaker, Accelerate and Icon showed highly significant values under different treatments. Traits such as germination percentage and survival percentage showed significant results for genetic advanced and heritability. Mean values of biochemical studies indicated that genotype "Accelerate" showed high mean values for antioxidant activity and total flavonoid content, while "Icon" showed significant results in phenolic content. Accelerate, Icon and Haymaker have reasonable variability and important mechanisms for conferring drought tolerance in alfalfa and ryegrass.

Keywords: alfalfa, rye grass, polyethylene glycol, drought stress, genetic advanced, heritability, phenotypic correlation and genotypic correlation

Abbreviations: PEG: polyethylene glycol, ANOVA: analysis of variance, GV: genotypic variance, PV: phenotypic variance, G.A: genetic advance, H²: heritability, DPPH: 2, 2-diphenyl-1-picrylhydrazyl.

Introduction

Alfalfa (*Medicago sativa* L.) is a perennial legume of great agronomical interest bearing high nutritional value for livestock and is one of major forage crop rich in

vitamins, minerals and phytonutrients (Xiao et al., 2015; Kumar et al., 2018). The deep root system of alfalfa helps to eliminate water contamination and can preclude soil erosion. Alfalfa is a leguminous crop that improves soil fertility. It fixes 135–605 kg/ha atmospheric nitrogen per year (Putnam et al., 2001). It is also an appropriate source for biofuel production to meet the energy demand of the increasing world population because of its perennial nature and rapid biomass accumulation. Alfalfa is also an important hay product which grows in arid and semi-arid regions and is beneficial because of its straightness and roots depth (Putnam et al., 2001).

Perennial ryegrass (*Lolium perenne* L.) is also an important crop because of CO₂ assimilation (Hu et al., 2013), and is the most frequently cultivated species considered properly for farming as well as forage production for livestock (Falloon and Betts, 2009; Furet et al., 2012). The growth rates of Ryegrass are very high with high nutritive values. However, the perennial ryegrass is a drought-susceptible grass species, although they seem to be a noticeable genotypic variation for drought stress responses (Turgeon, 2002).

Environmental factors daunting stress on plants, including drought, salinity, high and low temperature etc. cause extensive crop losses all over the world. In particular, the frequency of severe drought conditions is increasing due to constant increase in temperatures and the water scarcity (Flexas and Medrano, 2002; Hassine et al., 2010; Ghaderi et al., 2011). Geographic distribution and productivity of crop plant is limited by drought, which is a major abiotic stress, resulting in massive losses of yield (Farooq et al., 2009; Fang and Xiong, 2015; Cao et al., 2017). Among all abiotic and biotic stresses, drought is one of the major problems facing crop production in many regions of the world, where irregular summer drought periods along with warmer temperatures occur frequently (Schindler et al., 2009).

Due to drought stress water efficiency is reduced which reduces leaf size, stem extension and root proliferation (Ma et al., 2012). Drought also causes stomatal closure, membrane damage and altered functioning of enzymes as well as the CO₂ assimilation by leaves is hindered (Flexas and Medrano, 2002). Reactive oxygen species (ROS) are generated due to enhanced metabolite fluidity through the photo respiratory pathway which leads to increase oxidative load on the tissues which as a result severely damage the biological macromolecules (Zhu, 2002; Osakable et al., 2014). Due to an increase in consumption of water for various human activities water flow in the grassland's river is reduced 20–84% in the last 90 years (Schindler and Donahue, 2006).

Furthermore, United Nation's international panel on Climate change predicts, increased drought frequencies in many regions of the world in few decades (Stocker et al., 2013). These observations suggest that to ensure the sustainability of alfalfa and Ryegrass production, development of drought tolerant cultivars maybe necessary.

Shortage of water is a severe environmental restriction to plant productivity. In crop yield, losses exceed due to drought. Understanding of physiological mechanisms enables the plants to withstand against water shortage conditions. Due to various stresses including drought around the globe, the current production of fodder is not sufficient to fulfill needs of farmers and their livestock. It is also important for understanding the yield limiting factors under water deficit conditions. There is a need to screen the drought tolerant genotypes of alfalfa and rye grass to get the high yield and good quality of fodder. The present study was planned to screened alfalfa and rye grass genotypes, which could tolerate well under drought conditions at seedling stage.

Materials and methods

This experimental design was two factorial Completely Randomized Design with three replications. In this research work eight fodder genotypes (Ace, Hay maker, Cuf 101, Icon, Alfalfa, Alfalfa-1, Emerson and Accelerate) were used and summarized in *Table 1*. The first six genotypes were grouped in Alfalfa (*Medicago sativa* L.) crop and last two were grouped in Ryegrass (*Lolium*). Seeds were surface sterilized for 5 min with 10% Sodium hypochlorite solution and washed with distilled water. Five seeds of each genotype were placed on moist filter paper for each experimental unit with three replications. On the filter paper to provide proper moisture stress PEG solution treatments were applied for seed germination at 25 °C. Molecular weight of 6000 Polyethylene glycol (PEG-6000) was used as a drought stimulator. Three PEG stress were made viz., 5%, 10% and 15% of Polyethylene glycol⁻¹ 100 ml by dissolving in distilled water.

Table 1. List of genotypes of alfalfa and ryegrass

Sr. No.	Genotypes	Species
01	Ace	Alfalfa
02	Hay maker	Alfalfa
03	Cuf 101	Alfalfa
04	Icon	Alfalfa
05	Alfalfa	Alfalfa
06	Alfalfa-1	Alfalfa
06	Emerson	Ryegrass
08	Accelerate	Ryegrass

Description for treatment

Following treatments were used in this research project such as T₁ = Control (distilled water), T₂ = 5% PEG solution, T₃ = 10% PEG solution and T₄ = 15% PEG solution.

Seedlings traits

The data on following seedlings traits was collected during these studies such as Germination Percentage (%), Survival percentage (%), Coleoptile Length (cm), Shoot Length (cm), Root length (cm), Shoot fresh weight (g), Root fresh weight (g), Total fresh weight (g), Shoot dry weight (g), Root dry weight (g) and Vigor Index. Vigor index is calculated by given formula.

$$\text{Vigor index} = \text{Root length} + \text{Shoot length} \times \text{Seed germination (\%age)}$$

Bio-chemical studies

Following biochemical analysis were carried out.

Total phenolic contents (g)

By the method of (Waterhouse, 2002) the phenolic content in plant extracts was determined. By using 1 mg/ml of methanolic solution Phenolic content was determined.

The mixture contains 0.5 ml of methanolic solution of extract, 2.5 ml 7.5% NaHCO₃, 2.5 ml of 10% Folin-Ciocalteu's reagent and 5 ml of 10% Folin-Ciocalteu's reagent dissolved in water by mixing 0.5 ml methanol and 2 and 2.5 ml of 7.5% of NaHCO₃ blank solution was prepared. In a thermostat the samples were incubated at 45 °C for 45 min. By using spectrophotometer at 765 nm the absorbance was recorded.

Antioxidant activity (%)

Yu et al. (2002) determined the procedure of Antioxidant activity using stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. 0.25 mm solution of (DPPH) radical and different absorptions of ethanol (1 ml, 850 ml and 925 ml) was mixed with sample and kept in darkness for 30 min. Using spectrophotometer at 517 nm the activity was noticed and the conforming blank reading (control) was measured. The percentage of scavenging activity was determined as:

$$\text{Radical scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of sample}} \times 100$$

Total flavonoid (mg/100 g)

It was assessed by the method of (Chang et al., 2002). 10 ml of hot water and 0.1 M potassium acetate, 0.1 ml of aluminum chloride, 0.1 gram of fine sample was taken and mixed and 2.8 ml of distilled water was added subsequently. Activity was noted at UV-visible spectrophotometer at 415 nm after 30 min.

Statistical analysis

Analysis of variance (ANOVA) was conducting using computer software MSTATC (Bricker, 1991) means were calculated. Between the traits the Phenotypic and genotypic correlation coefficients were worked out (Snedecor, 1956). The linearly relationship between two quantitative variables is termed as correlation. The primary objective of correlation analysis is to measure the degree of linear association between two or more variables. On the other hand, regression analysis does not asses such kind of relationship. When the given variables are found to be related than we want to know how closely related they are. The estimation of genetic advance (ΔG) and heritability (h^2) at 5% selection intensity ($K = 2.06$) were obtained (Allard, 1960).

$$\sigma^2 g = \frac{MS_2 - MS_1}{r}$$

$$\sigma^2 p = \frac{MS_2 - MS_1}{R}$$

$$\sigma^2 e = MS \text{ error}$$

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

$$\Delta G = K \sqrt{\sigma^2 p} \times h^2$$

where: MS_2 = genotype mean square, h^2 = heritability, σ^2g = genotypic variance, σ^2p = phenotypic variance, r = number of replications, MS_1 = error means square, ΔG = genetic advance, K = selection intensity (2.06).

Results

Analysis of variance for various seedling traits of alfalfa and rye grass genotypes with artificial induce drought stress

Table 2 showed that analysis of variance (ANOVA) for germination percentage, shoot length, root length, total fresh weight, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, coleoptile length, survival percentage, and vigor index showed high significant differences for treatments and genotypes as well as for the interaction between treatment and genotypes, whereas total fresh weight and root fresh weight showed significance results for treatment and genotypes interaction and for treatments respectively. Table 2 consists of the mean square values of most of seedling traits of Alfalfa and ryegrass which performed well under different drought stress treatments which could be screened as drought resistant cultivars.

Table 2. Analysis of variance (mean squares values) for seedling traits with artificial induce drought stress in alfalfa and ryegrass

SOV	DF	GP	SL	RL	TFW	SFW	SDW	RFW	RDW	CL	SP	VI
T	03	3585.84**	549.15**	15.31**	8.80**	7.74**	1.82**	0.022*	1.59**	1.872**	7865.60**	11201.4**
G	07	747.01**	49.14**	19.76**	2.25**	3.42**	8.90**	0.001**	1.96**	0.186**	647.26**	865.2**
TXG	21	1163.38**	3.95**	3.44**	2.35*	1.84**	5.37**	0.001**	1.29**	0.361**	869.84**	1282.3**
Error	64	6.15	0.07	0.022	2.67	3.82	2.82	0.001	4.59	0.0087	9.17	22.7

DF: degree of freedom, SOV: source of variation, GP: germination percentage, SL: shoot length, RL: root length, TFW: total fresh weight, SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh weight, RDW: root dry weight, CL: coleoptile length, SP: survival percentage, VI: vigor index, T: treatments, G: genotypes, TXG: treatment interaction genotype

Estimation of genotypic correlation among different variables

The correlation values lie between -1 to +1. Shoot length had highly significant positive correlation with GP (0.50**) at genotypic level. In the given results, root length (RL), had highly significant positive relationship with germination percentage (GP) (0.67**) and shoot length (SL) (0.87**), which demonstrated a strong linear association and dependence of these traits and their movement in one coordination. On the other hand, total fresh weight (TFW) showed negative and significant correlation with GP (-0.086*), negative and non-significant with SL (-0.280), and positive non-significant with RL (0.100). Shoot fresh weight (SFW) showed negative and non-significant correlation with germination percentage (GP) (-0.41), and SL (-0.25), positive and non-significant with RL (0.14) and significant positive relationship with TFW (0.002*). Root fresh weight (RFW) had positive non-significant correlation with GP (0.29), SFW (0.71), and RL (-0.23), significant negative with SL (-0.44), and highly significant and positive with TFW (0.76*). Likewise, shoot dry weight (SDW) exhibited highly significant positive correlation with RFW (0.82**), significant positive with TFW (0.81*), and SFW (0.019*), non-significant positive with RL (0.067), negative and non-significant with GP (-0.10), and SL (-0.31). Root dry weight (RDW), showed non-

significant positive correlation with GP (0.12), and TFW (0.80), significant positive with SL (0.500*), RL (0.23*), and SFW (0.75*), highly significant positive correlation with RFW (0.81**), and SDW (0.87**) (*Table 1*). The CL showed highly significant positive correlation with RL (0.68*), and positive significant correlation with SL (0.66*). Likewise, significant correlation was shown by SP with GP (0.83*), and SDW (0.24*). At genotypic level the VI showed highly significant correlation with SP (0.56**), significant positive correlation with CL (0.48*), SFW (0.07*), and GP (58*) as shown in *Table 3*.

Table 3. Estimates of genotypic correlation coefficients among alfalfa and rye grass genotypes

Variables	G.P	S.L	R.L	T.F.W	S.F.W	R.F.W	S.D.W	R.D.W	C.L	S.P	V.I
G.P	1										
S.L	0.507**	1									
R.L	0.674**	0.875**	1								
T.F.W	-0.086*	-0.280	0.100	1							
S.F.W	-0.141	-0.255	0.143	0.002*	1						
R.F.W	0.294	-0.445*	-0.233	0.768**	0.718	1					
S.D.W	-0.100	-0.318	0.067	0.818*	0.019*	0.824**	1				
R.D.W	0.124	0.500*	0.232*	0.803	0.750*	0.818**	0.875**	1			
C.L	-0.069	0.664*	0.686**	-0.100	-0.106	-0.204	-0.142	-0.217	1		
S.P	0.830*	-0.786*	-0.704*	0.247	0.211	0.423	0.240*	0.279	-0.239	1	
V.I	0.582*	0.008	0.040	-0.080	0.076*	-0.017	0.131	-0.416	0.486*	0.567**	1

Phenotypic correlation between alfalfa and rye grass genotypes

The genotypic correlation between variables of alfalfa and rye grass genotypes is presented in *Table 4*. It is evidenced from the *Table 4*, that SL had non-significant negative correlation with GP (-0.50). Likewise, RL showed negative and highly significant correlation with GP (-0.66**), and positive non-significant with SL (0.87). TFW showed negative and non-significant correlation with GP (-0.08), and SL (-0.27). In the same way, SFW showed negative non-significant correlation with GP (-0.14), SL (-0.25), significant positive with RL (0.14), and highly significant positive with TFW (0.99**). RFW exhibited positive and significant correlation with GP (0.27*), highly significant positive correlation with TFW (0.72**), and SFW (0.65**), negative and non-significant correlation with SL (-0.41), and RL (-0.21). In the same way, SDW showed highly significant positive correlation with TFW (0.99**), SFW (0.99**), and RFW (0.71**), non-significant positive correlation with RL (0.66), and non-significant negative correlation with GP (-0.09), and SL (-0.30). RDW showed positive and non-significant correlation with GP (0.09), RL (0.20), and SFW (0.67), negative and significant correlation with SL (-0.44), highly significant and positive correlation with TFW (0.72**), RFW (0.92**), and SDW (0.74**). At phenotypic level CL showed highly significant positive correlation with SL (0.65**), and RL (0.67**), and significant positive correlation with SFW (0.10*). On the other hand, SP showed significant positive correlation with SL (0.78*), RL (0.69*), TFW (0.24*), and SDW (0.23*) (*Table 2*). The vigor index showed highly significant positive correlation with SP (0.55**), and GP (57**).

Table 4. Estimates of phenotypic correlation coefficients among alfalfa and rye grass genotypes

Variables	G.P	S.L	R.L	T.F.W	S.F.W	R.F.W	S.D.W	R.D.W	C.L	S.P	V.I
G.P	1										
S.L	-0.503	1									
R.L	-0.668**	0.874	1								
T.F.W	-0.083	-0.279	0.100	1							
S.F.W	-0.144	-0.254	0.142	0.994**	1						
R.F.W	0.278*	-0.411	-0.217	0.721**	0.653**	1					
S.D.W	-0.096	-0.304	0.066	0.996**	0.990**	0.719**	1				
R.D.W	0.090	-0.445*	0.208	0.724**	0.672	0.924**	0.745**	1			
C.L	-0.063	0.653**	0.674**	-0.015	0.107*	-0.169	-0.104	-0.186	1		
S.P	0.821	0.780*	0.696*	0.2485*	0.207	0.395	0.232*	0.240	-0.229	1	
V.I	0.572**	0.007	0.039	-0.0810	-0.076	-0.148	-0.138	-0.358	0.483*	0.557**	1

Heritability and genetic advance

The results indicated that all of the given traits had higher estimates of heritability. As per results the values for genotypic advance for given traits are, higher genetic advance for VI (27.25), SDW (26.1), SL (25.16), GP (22.35), and SP (21.89), moderate genetic advance for CL (14.89), and RDW (11.8), low genetic advance for RFW (9.1), SFW (4.3), and TFW (3.1). The estimates of heritability were found higher for all traits viz., SL (99), RL (99), TFW (99), SFW (99), VI (98), GP (98), SP (98), SDW (96), RFW (85), and RDW (80) (Table 5). The values of GV and PV are given in Table 5.

Table 5. Estimates of heritability and genetic advance

Traits	GV	PV	H ²	G. A
GP	9.970	10.02	98	22.3
SL	30.09	30.92	99	25.16
RL	30.8	30.82	99	2.64
TFW	21.57	21.63	99	3.1
SFW	25.15	25.63	99	4.3
RFW	11.75	12.71	85	9.1
SDW	22.23	22.65	96	26.1
RDW	9.43	10.52	80	11.8
CL	7.08	7.18	97	14.89
SP	14.94	15.09	98	21.89
VI	10.52	10.59	98	27.25

GV = genotypic variance, PV = phenotypic variance, G.A = genetic advance, H² = heritability

Biochemical traits

Total phenolic contents (mg GAE/g)

Mean values for total phenolic contents were shown in Figure 1. Means ranged from 2.14-2.96%. Highest value was shown by icon (2.96%). Whereas alfalfa1 (2.83%) and Emerson (2.68%) were showed good phenolic content while lowest value was shown by ace (2.14). It was estimate total phenolics content that was present in different fodder

genotypes as they have redox abilities and antioxidant properties. Therefore, they act as a reducing agent. Phenolic compound is responsible for taste, appearance of cereals grains and color such in wheat. In plants disease resistance and defense against microorganism is due to Phenolic compound.

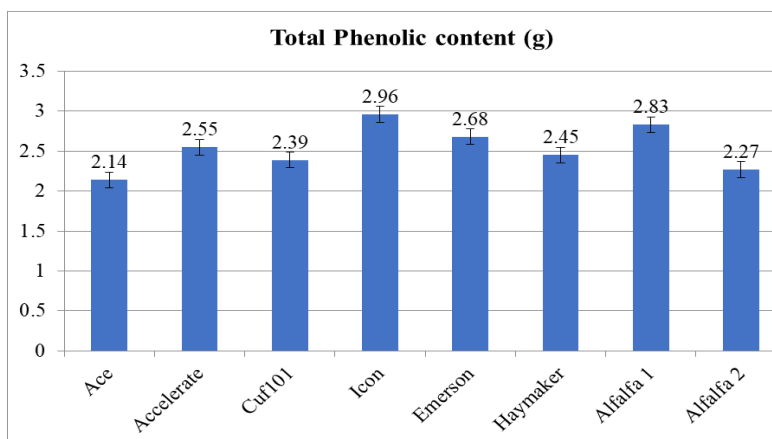


Figure 1. Mean values for total phenolic content in alfalfa and rye grass genotypes

Antioxidant activity DPPH (%)

The results regarding antioxidant activity of methanolic extract were shown in Figure 2. Antioxidant activity varied from 42.4-80.3% in alfalfa and rye grass genotypes. Highest antioxidant activity was observed in case of genotype alfalfa 2 (80.3%). Significant antioxidant activity was also noted in accelerate (78.56%) and haymaker (71.45%) while lowest value was observed for ace (42.4%). On spectrophotometer at 517 nm all readings were taken for all genotypes. Free-radical scavengers were tested by using DPPH radical to check the ability of different compounds.

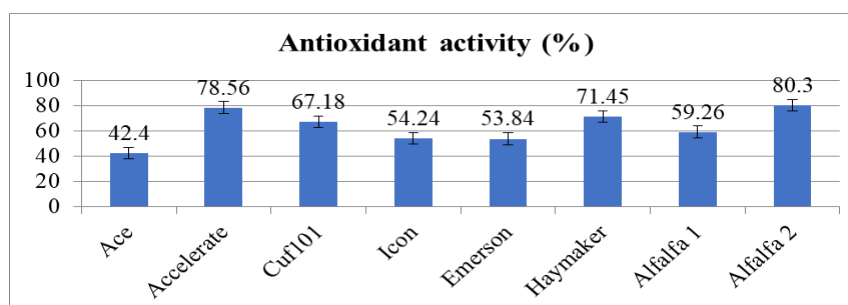


Figure 2. Mean values for antioxidant activity in alfalfa and rye grass genotypes

Total flavonoids content (mg/100 g)

Total flavonoids content was determined by using aluminum chloride. The mean values for flavonoid content varied from 1.06-2.61% in alfalfa and rye grass genotypes. In Figure 3 highest flavonoid content was observed in case of genotype accelerate (2.61%) and significant flavonoids content observed in cuf 101 and icon (2.38%) while

lowest was observed for alfalfa 1 (1.06%). The most common flavonoid that present in most of the food and extensively distributed in nature was Quercetin used as a standard. It has antioxidant ability and antiviral characteristics. These studies indicated that rye seed extract has a significant source of antioxidant which may well linked with its potential use as a functional food.

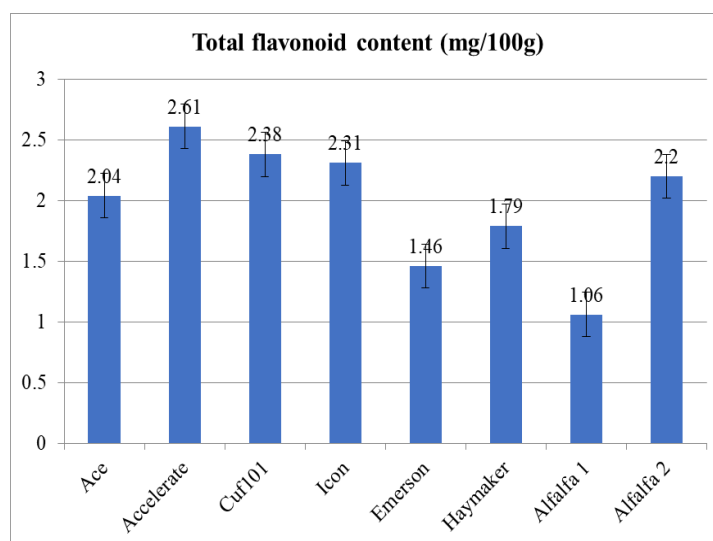


Figure 3. Mean values for total flavonoid in alfalfa and rye grass genotypes

Discussion

Alfalfa and Ryegrass are the main source of starch and energy and offer considerable amounts of a number of products which are vital or beneficial for health, notably protein, vitamins (particularly B vitamins), dietary fiber, and phytochemicals for animals (Igrejas and Branlard, 2020). It is contributing the trade and highest variety of important food derivatives (Igrejas and Branlard, 2020). Likewise, alfalfa is one of the most significant legume crops and the most significant use of alfalfa is in the dairy industry. Alfalfa is the premier forage for dairy cows. Thus, a large constituent of the high-quality dairy harvests like, milk, yogurt, cheese, cream, should be attributed to the role of alfalfa and Ryegrass to dairy rations (Glagoleva et al., 2021). By keeping this in mind, it is important to breed the alfalfa and ryegrass genotypes for high quality food items and to step forward for sustainable economy (Glagoleva et al., 2021). The aim of study was to investigate the inter relationship among different variables of Ryegrass and alfalfa genotypes.

The genotypes were evaluated and data was recorded against several key agronomic traits. The genetic association enables forecasts of selection response in one or more characters to be made when assortment is another trait or traits (Abdulhamed et al., 2021). Our results concerning the relationship of key traits and their use in selection programs. Among several studies traits, the highly significant positive correlation of SL with GP (0.50**) at genotypic level demonstrated that these two traits are highly dependent on each other and selection of these two variables would lead to increase in yield of the Ryegrass and alfalfa genotype. The genotypic correlation among the traits is shown in Table 2, however the correlation of SP with GP (0.83*) is highly significant which is an indication of the yield improvement by direct selection. Meanwhile, the VI

which is the supreme index of seed quality, and determines the potential for rapid emergence of plants (Zhao et al., 2021). It also indicates the seed's ability to produce normal seedlings subjected to less optimum growth conditions (Khumalo et al., 2021). VI showed significant positive correlation with GP (0.58*), SFW (0.07*), CL (0.48*), and highly significant positive correlation with SP (0.56**) at genotypic level which depicted that by increasing these variables the seed vigor index of Ryegrass and alfalfa genotypes would be increased.

Phenotypic relationship is an essential parameter that helps to select the traits using the traditional way, which needs individual-level phenotypic statistics (Zhang et al., 2018). A phenotypic relationship is the relationship between records of two characters on the same plant and is usually assessed by the product-moment correlation number (Zhao et al., 2021). A phenotypic feature is a noticeable, observable, and quantifiable trait; it is the expression of genes in an apparent way. The phenotypic correlation between the traits is a sign of indication of environmental influence on traits phenotype (Poudel et al., 2021; Wang et al., 2021). Our results showed the significant positive correlation of SP with SL (0.78*), RL (0.69*), TFW (0.24*), and SDW (0.23*) at phenotypic level. In the same way the highly significant positive correlation of VI with SP (0.55**) which revealed the effective selection plan of genotypes for on the basis of these traits as shown in *Table 3*. The selection of genotypes of Ryegrass and alfalfa traits would be rewarding on the basis of the correlation among the given traits. Higher phenotypic and genotypic variance is the base for existence of plants in nature and for crop improvement. Variation in plant genetic resources offers chance for plant breeders to breed new and better cultivars with required characteristics, which comprise farmer-preferred traits like, high yield potential, large seed, etc. (*Table 3*). (Kumar et al., 2022; Long et al., 2022). The traits had higher variance in the proposed study are leading towards key selection plan for development of better genotypes.

Heritability is a statistic used in the fields of breeding and genetics that guesses the degree of difference in a phenotypic trait in a progeny that is due to genetic variation among individuals in that progeny (Juraev et al., 2021). Genetic advance describes the degree of gain attained in a character under a specific selection pressure. High genetic advance together with high heritability offers the most appropriate condition for selection (Gebremariam et al., 2022; Hossain et al., 2021). It is obvious from the results that all of the given traits of Ryegrass and alfalfa genotypes showed higher heritability and differential pattern of genetic advance. GP had higher heritability (98) with higher genetic advance (22.3) which showed that selection of genotypes on the basis of GP would lead to development of genotypes with higher GP ratio. Following this pattern, VI also showed higher heritability (98) with higher GA (27.25) which showed the genetic gain from selection. The higher estimates of heritability coupled with higher genetic advance was also observed for SDW (96, 26.1), SP (98, 21.89), and SL (99, 25.16). These results showed the involvement of additive gene action and improvement in average genetic value of trait in a population or average improvement in phenotype due to selection in a progeny after breeding cycles (Yadesa et al., 2022).

For most of the variables assessed in this study, there were highly significant variances between genotypes, demonstrating that there are plenty of genetic differences among genotypes. The difference in extent of phenotypic and genotypic difference among all features are recommended for genotypes which played a role in traits

expression as shown in *Table 4*. As a result, the enormous amount of difference is owed to a variety of material sources as well as other effects. Earlier, Poudel et al. (2021) showed similar pattern of results and validated the current findings. Further examination of these genotypes are recommended to validate the positive outcomes found in this study and to get more appreciated and reliable outcomes. Finally, it is possible that the information gathered during this analysis will be valuable to breeders working to develop high-yielding, genotypes (Poudel et al., 2021). Easwar and Viswanatha (2020), worked by using DPPH method for estimation of antioxidant of different glycine max cultivars. The presence of high content of phenolic which are well potential source of antioxidants and free radical scavengers due to antioxidant activity. DPPH scavenging activity of soybean leaf extracts is recognized and inhibits lipid peroxidation through degrading the radicals. Our results showed accordance with their results. They were used to evaluate the antioxidant activity of food and different plant extract (Lu et al., 2012). The presence of high content of phenolics and flavonoids particularly quercetin which are well potential source of antioxidants and free radical scavengers present in seed of ethanolic extracts of rye grass and alfalfa may be recognized which inhibited lipid peroxidation through degrading the radicals. Similar work was done by Wang et al. (2020), they estimated the total flavonoid contents in wheat grain as in outer layer of grain they found that flavonoid content was higher. Our results showed difference to their results may be due to different genetic material, protocols and methods that has been used. Accelerate showed maximum total flavonoid content could be our target for fodder genotypes improvement program regarding breeding for improved nutritional value.

Conclusion and recommendation

From overall performance it is observed that analysis of variance for physiochemical and seedling traits showed the effect of treatments on genotypes for germination %age, survival percentage, shoot and shoot length (cm), fresh and dry root weight (g), fresh shoot weight (g) shoots dry weight (g), total fresh weight, vigor index and proline content was highly significant. On the basis of mean following genotypes showed high values under different treatments as named Haymaker, Accelerate and Icon. Following traits such as germination percentage and survival percentage showed significant results for genetics advanced and heritability. Length of shoot and root, germination %age, total fresh weight, shoot fresh weight, coleoptiles length, survival percentage and vigor index demonstrate high and positive relation for genotypic and phenotypic correlation. In biochemical analysis Accelerate showed high mean values for antioxidant activity and total flavonoid. Icon showed good results in phenolic content. Accelerate, Icon and Haymaker have reasonable variability and could be subjected in future breeding programs and might be used in areas where drought condition occurs.

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