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Trait association and path value analysis of seed germination parameters of Kalmegh (*Andrographis paniculata* Wall. Ex Ness) germplasm

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Abstract
Keeping the importance of screening of associated factors for improved seed germination, an experiment was undertaken with 25 germplasm of Kalmegh at BAU, Kanke to study its seed germination behavior suited for the climatic and edaphic conditions of Jharkhand. Correlation matrix between seed germination parameters of Kalmegh seeds under field condition indicated that the germination energy had highly significantly positively correlation with germination percentage (0.939) and seedling vigour index (0.914). Similarly, germination percentage had highly significantly correlation with seedling vigour index (0.970). Multiple regression analysis of germination parameters indicated that germination period, rate of germination, germination percentage, shoot as well as root length of seedlings and root-shoot ratio affect seedling vigour index of Kalmegh significantly. Path analysis of characters influencing seedling vigour index of Kalmegh seedlings under field conditions showed that eight parameters had direct positive effect on seedling vigour index, in which maximum direct effect was observed by germination percentage (0.716). Three parameters showed direct negative effect on seedling vigour index, in which maximum impact was shown by days taken for complete of seed germination (-0.235). From the findings, it can be inferred that for higher seedling vigour index, rate of germination, germination percentage, shoot as well as root length of seedlings and root-shoot ratio of seedlings should be selected as important parameters as their higher values gave higher seedling vigour index.

Keywords: Kalmegh, *Andrographis paniculata,* seed germination, correlation, path analysis, germination percentage, seedling vigour index

Introduction
Germination of a seed is the emergence and development of a seedling to a stage where the aspect of its essential structures (root system, shoot axis, cotyledons, terminal buds) indicates whether or not it is able to develop further into a satisfactory plant under favorable soil conditions (ISTA, 2006) [7]. Since germination is a complex biological process and at a point of time several factors have to enact simultaneously the resultant effect is reflected, in the form of emergence of seedling, after a certain period of time (Black and Halmer, 2006) [3]. Effective stand after germination (associated parameters like germination energy, germination period, germination percentage etc.) is important characteristic that gives an idea about the final population. Thus, it would be desirable to have information regarding these parameters for producing good quality seed in case of Kalmegh (Kumar *et al.*, 2011) [11]. Therefore, enhancing seed germination to produce healthy and vigorous seedlings is crucial (Kohli & Kumari, 1986; Kumari *et al.*, 2012) [12, 10]. The positive correlation between desirable

characters is favorable for simultaneous improvements of associated characters. The estimate of correlation coefficient mostly indicate interrelationship of different characters but it does not furnish information on cause and effect. Under such situation path analysis helps the breeder to identify the index of selection.

A systematic study on seed germination parameter is essential for any seed propagated crop to ensure crop stand and yield. Since Kalmegh is seed propagated, it is essential to assess the seed quality for ensuring the crop stand and herb yield which depend on quality seed (Kumar *et al.*, 2011) [11]. Germination energy is the measure of the speed of germination and hence responsible for the vigor of the seed and of the seedling which it produces. Seed vigor is a measure of the quality of seed, and involves the viability of the seed, the germination percentage, germination rate and the strength of the seedlings produced. The period during which maximum number of seedlings could be obtained is called as germination period (Czabator, 1962) [4].

Keeping in view the importance of associated factors for improved seed germination of Kalmegh germplasm, a systematic research trial was undertaken to screen out the factors impart maximum contribution towards higher seed germination at Birsa Agricultural University, Ranchi under the climatic and edaphic conditions of Jharkhand. *Andrographis paniculata* is a member of the Acanthaceae family, belongs to small tribe *Andrographideae* of the subfamily *Acanthoideae*. Kalmegh is an important indigenous medicinal plant commonly known as 'King of Bitters' (Gomathinayagam *et al*., 2009) [1] found throughout tropical & sub-tropical Asia (Kataky and Handique, 2010) [2]. Kalmegh can be grown by many methods of cultivation such as sowing seeds in rows, broadcasting or transplanting seedlings (Department of Medical Sciences, 1999) [5]. The seeds are sown during May-June and the seedlings are transplanted at a distance of 60 cm x 30 cm. Low seed germination is one of the main problems in Kalmegh because of dormancy (Saraswathy *et al.*, 2004; Kumar *et al.*, 2011b; Talei *et al.*, 2012) [15, 8, 16].

Materials and Methods

Location of the experimental site: The experimental site was located at Research Farm, Birsa Agricultural University, Kanke, Ranchi, located in the plateau region of Jharkhand. Geographically, it is located at $23^0$26'30" N latitude and $85^0$18'20" E longitude in Chhotanagpur plateau, situated in north eastern part of India and at an altitude of between 646 m above the mean sea level. The soil of the site is lateritic, developed from granite-gneiss, sandy loam in texture, sedentary in nature and well drained with low water holding capacity and poor consistency.

Climatic conditions of the experimental site: The general climate of the region is classified as 'sub humid mega thermal' with mean daily temperature of about 24.2 ^{O}C. The region is characterized by three distinct seasons namely summer, rainy and winter seasons. The mean relative humidity is about 70.88% with its range from 57.0 to 92.0% in the area. The monsoon breaks out in the middle of June and last till mid-October. The average annual rainfall of this area is approximately 1400mm which is mostly erratic, punctuated with occasional dry spells. The mean wind velocity and evaporation varies from 3.6 to 4.4 km/hr and 130 to 140 mm, respectively.

Experimental materials: The experimental materials comprised of twenty five genotypes of Kalmegh, for which seeds were collected from its natural habitat across six states of India and NBPGR, New Delhi including wild and cultivated varieties. Out of 25 Kalmegh accessions, 4 each were collected from Jharkhand, Chhattisgarh, Madhya Pradesh, Karnataka, 6 from Orissa, 2 from NBPGR and one from Gujarat. Collected seed samples were germinated, raised in polytubes and maintained under identical growing conditions in experimental area.

Table 1: Details of *Andrographis paniculata* germplasm used as experimental materials

Sl. No.	State/ Institution	Germplasm	Status
1.	Jharkhand	T_1 - $JHAP_1$, T_2 - $JHAP_2$, T_3 - $JHAP_3$, T_4 - $JHAP_4$	Wild
2.	Orissa	T_5 - OAP_1, T_6 - OAP_2, T_7 - OAP_3, T_8 - OAP_4, T_9 - OAP_5, T_{10} - OAP_6	Wild
3.	Chhattisgarh	T_{11} - $CHAP_1$, T_{12}- $CHAP_2$, T_{13} - $CHAP_3$, T_{14} - $CHAP_4$	Wild
4.	Madhya Pradesh	T_{15} - $MPAP_1$, T_{16} - $MPAP_2$, T_{17} - $MPAP_3$, T_{18} - $MPAP_4$	Wild
5.	Karnataka	T_{19} - KAP_1, T_{20} - KAP_2, T_{21} - KAP_3, T_{22} - KAP_4	Wild
6.	NBPGR, New Delhi	T_{23} - IC 111286, T_{24} - IC 471890	Cultivated
7.	Gujarat	T_{25} - GAP_1	Wild

The germination study of all the twenty five germplasm of *Andrographis paniculata* was undertaken at field level in polythene tubes (13 cm × 7 cm) by establishing nursery. Experiment was conducted in Completely Randomized Design with 25 treatments replicated thrice by following the procedure outlined by Panse and Sukhatme (1985) [14]. Number of polytubes kept in a treatment was 50 and in each polytubes two seeds of Kalmegh were sown. Finely powdered, sieved farm yard manure, sand and soil in the ratio of 1:2:1 was taken and filled into poly tubes. The mixture was treated with carbendazim (0.2%) before seed sowing. Plants were irrigated regularly on alternative days except during the rainy day. Data on germination trend was recorded from initiation to completion of germination (Kumar *et al.*, 2010) [9] and based upon this different germination parameters was calculated. Parameters studied were days taken for initiation of seed germination, days taken for 50% of final seed germination, days taken for completion of seed germination, germination period (days), rate of germination, germination energy (%), germination percentage (%), shoot and root length (cm) of seedlings at time of transplantation, root-shoot ratio of seedlings, seedling vigour index and 100 seed weight (g) and calculated by using the formula cited by Czabator (1962) [4].

Correlation is a measure of association between two variables. The correlation coefficient between characters was calculated by using the formula suggested by Miller *et al.*, (1958) [13]. Multiple regression analysis involving more than one independent variable and when all the independent variable is assumed to affect the dependent variable in a linear fashion and independently of one another, determines the nature and strength of the relationship between characters. Path coefficient analysis is a standardized partial regression coefficient and measures the direct influence of one variable upon another and permits the separation of correlation coefficient into components of direct and indirect effects. The

path analysis helps to resolve the correlation further and throws more light on the way in which component traits contribute towards specifically identifying important component traits. It is calculated by using the methodologies given by Dewey and Lu (1959) [6].

Results and Discussion

Table 2 represents correlation matrix between seed germination parameters of Kalmegh seeds under field conditions. Days taken for completion of seed germination was found highly positively correlated with germination period but highly negatively correlated with rate of germination of seeds (-0.979).

Table 2: Correlation matrix between germination parameters of *Andrographis paniculata* seeds under field conditions

Variables	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}
V_2	-0.350^{NS}	1.000									
V_3	0.036^{NS}	0.526**	1.000								
V_4	-0.039^{NS}	-0.296^{NS}	0.193^{NS}	1.000							
V_5	-0.177^{NS}	0.153^{NS}	0.455*	0.891**	1.000						
V_6	0.011^{NS}	0.224^{NS}	-0.243^{NS}	-0.979**	-0.904**	1.000					
V_7	0.230^{NS}	-0.324^{NS}	-0.370^{NS}	0.156^{NS}	0.007^{NS}	-0.161^{NS}	1.000				
V_8	0.285^{NS}	-0.338^{NS}	-0.338^{NS}	0.293^{NS}	0.135^{NS}	-0.305^{NS}	0.939**	1.000			
V_9	0.270^{NS}	-0.458*	-0.465*	0.226^{NS}	0.023^{NS}	-0.232^{NS}	0.678**	0.724**	1.000		
V_{10}	0.397*	-0.391^{NS}	-0.427*	0.155^{NS}	-0.046^{NS}	-0.175^{NS}	0.688**	0.744**	0.756**	1.000	
V_{11}	-0.085^{NS}	0.409*	0.289^{NS}	-0.043^{NS}	0.126^{NS}	0.035^{NS}	-0.299^{NS}	-0.300^{NS}	-0.653**	-0.112^{NS}	1.000
V_{12}	0.317^{NS}	-0.360^{NS}	-0.334^{NS}	0.266^{NS}	0.096^{NS}	-0.282^{NS}	0.914**	0.970**	0.820**	0.852**	-0.331^{NS}

*- significant at 5% level, **- significant at 1% level, R-square value: 0.9972

Where V_1 - Seed weight, V_2 - Days taken for initiation of seed germination, V_3 - Days taken for 50% of final seed germination, V_4 - Days taken for completion of seed germination, V_5 - Germination period, V_6-Rate of germination, V_7 - Germination energy, V_8 - Germination percentage, V_9 – Shoot length of seedlings at transplantation stage, V_{10} – Root length of seedlings at transplantation stage, V_{11} – Root-shoot ratio of seedlings at transplantation stage, V_{12} – Seedling vigour index of seedlings at transplantation stage.

Germination period of seeds was found highly negatively correlated with rate of germination (-0.904). Germination energy of seeds was found highly significantly positively correlated with germination percentage (0.939), shoot length of seedlings (0.678), root length of seedlings (0.688) and seedling vigour index of seedlings at transplantation stage (0.914). Similarly, germination percentage of seeds was found highly significantly correlated with shoot length of seedlings (0.724), root length of seedlings (0.744) and seedling vigour index (0.970). Shoot length of seedlings was found highly positively correlated with root length of seedlings (0.756) and seedling vigour index (0.820), but highly negatively correlated with root-shoot ratio of the seedlings. The root length of seedlings was found highly positively correlated with seedling vigour index (0.852), but root-shoot ratio had non-significant correlation with it. Talei *et al.*, (2012) [16] also observed significant correlation between germination traits of Kalmegh seeds.

Table 3 represents the regression coefficients and significance of germination parameters affecting seedling vigour index of Kalmegh seedlings under field conditions. Perusal of data indicated that rate of germination of the seeds had highly significant effect on seedlings vigour index.

Table 3: Regression coefficients and significance of germination parameters affecting Seedling vigour index *Andrographis paniculata* seedlings under field conditions

Variables	Coefficients	Standard Error	t-value
V_1	-2,071.212	585.325	-3.539
V_2	-90.443	41.671	-2.170
V_3	90.513**	15.007	6.031
V_4	-74.533	50.489	-1.476
V_5	57.774**	42.817	1.349
V_6	665.469**	1,616.131	0.412
V_7	0.094	2.363	0.040
V_8	16.220**	1.280	12.668
V_9	71.891**	21.005	3.423
V_{10}	82.354**	14.957	5.506
V_{11}	21.750**	57.774	0.376
Constant : -1,471.868			

**- significant at 1% level, $t_{(10)}$ at 5% = 2.228, $t_{(10)}$ at 1% = 3.169

Further the germination parameters like days taken for 50% of final seed germination, germination period, germination percentage, shoot as well as root length of seedlings and root-shoot ratio affect seedling vigour index of Kalmegh significantly, remaining parameters do not show any significant effect.

Table 4 represents path analysis of characters influencing seedling vigour index of Kalmegh seedlings under field conditions. Eight parameters show direct positive effect on seedling vigour index, in which maximum direct effect was observed by germination percentage (0.716) followed by root length of seedlings (0.264) while minimum direct effect was showed by germination energy (0.002) and root-shoot-ratio of

seedlings (0.014). Three parameters showed direct negative effect on seedling vigour index, in which maximum impact was shown by days taken for complete of seed germination (-0.235) and minimum by seed weight (-0.079). Among the indirect effect, the maximum positive indirect contribution of days of seed germination completion was noticed through germination percentage (0.210) and the maximum indirect negative impact through rate of germination (-0.035). The germination period showed maximum indirect positive effect through germination percentage and maximum indirect negative impact through days taken for completion of seed germination (-0.209). Rate of germination of seeds showed profound negative indirect impact through germination percentage (-0.219) and germination period (-0.166); but it showed high indirect positive impact through days taken for completion of seed germination (0.230). Germination energy of seeds contributed positively through indirect effect on germination percentage (0.673). Germination percentage of seeds showed indirect positive impact through shoot length (0.141) and root length (0.197) of seedlings. Maximum indirect positive impact of shoot length of seedlings was noticed through germination percentage (0.519), and maximum indirect positive impact of root length was also observed through germination percentage (0.533). Profound negative indirect impact of root-shoot ratio of seedlings was noticed through germination percentage (-0.215) and shoot length of seedlings (-0.217). From the above findings, it can be inferred that for higher seedling vigour index, rate of germination, germination percentage, shoot as well as root length of seedlings and root-shoot ratio of seedlings should be selected as import and parameters as their higher values gave higher seedling vigour index.

Table 4: Path analysis of characters influencing seedling vigour index of *Andrographis paniculata* seedlings under field conditions

Variables	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	Correlation coefficient
V_1	-0.079	0.052	0.006	0.009	-0.032	0.000	0.000	0.204	0.052	0.105	-0.001	0.317
V_2	0.028	-0.148	0.084	0.070	0.028	0.008	-0.001	-0.242	-0.089	-0.103	0.006	-0.360
V_3	-0.003	-0.078	0.159	-0.045	0.083	-0.009	-0.001	-0.242	-0.090	-0.113	0.004	-0.344
V_4	0.003	0.044	0.031	-0.235	0.163	-0.035	0.000	0.210	0.044	0.041	-0.001	0.266
V_5	0.014	-0.023	0.072	-0.209	0.183	-0.032	0.000	0.096	0.005	-0.012	0.002	0.096
V_6	-0.001	-0.033	-0.039	0.230	-0.166	0.036	0.000	-0.219	-0.045	-0.046	0.001	-0.282
V_7	-0.018	0.048	-0.059	-0.037	0.001	-0.006	0.002	0.673	0.132	0.182	-0.004	0.914
V_8	-0.023	0.050	-0.054	-0.069	0.025	-0.011	0.002	0.716	0.141	0.197	-0.004	0.970
V_9	-0.021	0.068	-0.074	-0.053	0.004	-0.008	0.001	0.519	0.194	0.200	-0.009	0.820
V_{10}	-0.031	0.058	-0.068	-0.036	-0.008	-0.006	0.001	0.533	0.147	0.264	-0.002	0.852
V_{11}	0.007	-0.061	0.046	0.010	0.023	0.001	-0.001	-0.215	-0.127	-0.030	0.014	-0.331

Residual-0.0028

Conclusion

The correlation matrix between germination parameters of Kalmegh seeds showed that days taken for completion of seed germination and germination energy had highly positively correlation with germination percentage. Multiple regression analysis also showed germination energy had highly significant effect on germination percentage of Kalmegh seeds. Path analysis of germination parameters showed direct positive effect of germination period, rate of germination and germination energy on germination percentage of Kalmegh seeds. Perusal of date indicated that germination energy is the most critical factor to obtain higher germination percentage of Kalmegh seeds.

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