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Assessing the Stability of Root Yield and Biochemical Traits in Ashwagandha [*Withania somnifera* (L.) Dunal]

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ABSTRACT

This study investigates the genetic diversity and stability of ashwagandha (*Withania somnifera*) crosses through field trials conducted across three diverse environments. A comprehensive evaluation of four traits was performed where 60 crosses were studied, certain combinations exhibited exceptional stability and performance, particularly in dry root yield and total alkaloid content. Crosses such as UWS-92 x UWS-10, UWS-100 x UWS-10, UWS-104 x UWS-10 and GP-51 x UWS-10 consistently demonstrated regression coefficients below unity ($b^{I}<1$) for dry root yield, indicating superior stability and productivity under adverse conditions. Similarly, crosses like UWS-131 x UWS-60 and UWS-92 x UWS-10 exhibited outstanding stability with total alkaloid content, surpassing the average mean of all crosses. These findings highlight the potential of specific cross combinations to enhance ashwagandha cultivation resilience and optimize key economic traits. The study underscores the significance of stable crosses for improving dry root yield and alkaloid content, contributing to the sustainability and profitability of ashwagandha cultivation.

***Key words**: Ashwagandha; Eberhart-Russell stability model; Dry root yield and Total alkaloid (%) content.*

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Introduction

Ashwagandha, also known as Indian ginseng or winter cherry, has gained recognition for its adaptogenic properties which are believed to help the body cope with stress and promote overall wellbeing (Mikulska *et al*., 2023). Furthermore, its diverse therapeutic applications encompass anti-inflammatory, anti-cancer and neuro protective effects contributing to its increasing popularity in global herbal medicine markets (Williamson *et al*., 2020; Ahmad *et al*., 2017). Indian roots exhibit a diverse range of alkaloids with reported contents ranging from 0.13% to 0.31%, although higher yields have been observed in other regions. Among the identified alkaloids are cuscohygrine, tropine and withananine, alongside amino acids like aspartic acid and tyrosine. As the global interest in herbal medicine continues to rise, Ashwagandha emerges as a valuable botanical resource, poised to make a substantial impact on both traditional and contemporary healthcare practices. Different genotypes respond differently to environmental conditions, highlighting the importance of genotype-environment interactions in influencing crop yields. Understanding these interactions is crucial for developing well-adapted crop varieties. In the case of Ashwagandha, stable hybrids are currently unavailable, emphasizing the need for research in this area. Identifying stable and adaptable genotypes across diverse conditions is essential for crop improvement. Selecting genotypes with reduced sensitivity to specific environments can enhance overall crop resilience and productivity. This study contributes to addressing this gap by examining stability and adaptability in Ashwagandha genotypes.

Materials and Method

The experimental setup consisted of 60 crosses which were created by L x T at Instructional Farm, Rajasthan College of Agriculture, MPUAT, Udaipur by using 20 lines and 3 testers in Rabi 2021-2022. The crosses were evaluated in randomized block design with three replications across three different locations *viz*., Instructional Farm, Rajasthan College of Agriculture, MPUAT, Udaipur; Agricultural Research Station, Banswara and Krishi Vigyan Kendra, Chittorgarh in Rabi 2022-23. The spacing between rows and plants was maintained at 30 cm and 10 cm, respectively. Observations were made for various traits including root length per plant (cm), number of secondary and tertiary roots per plant, dry root yield (g/plant) and total alkaloid (%) content.

Results and Discussion

The results from Table 1 revealed significant differences among genotypes for all four quantitative traits across each environment, as indicated by the highly significant mean sum of squares attributed to genotypes (G) for all traits. Moreover, genotypic interactions with the environment (G x E) were also significant across all traits. The environmental effects (linear) were significant for all traits except total alkaloid (%) content. Notably, the interaction between genotype and environment (G x E, linear) was significant for root length, dry root yield and total alkaloid (%) content. These findings underscore substantial genetic diversity among the genotypes, highlighting the potential to identify stable hybrid combinations through selective breeding. Lal (2015), Kumar *et al*. (2020), Yadav *et al*. (2024) and Ahmed *et al.* (2024) have all reported significant differences among genotypes and genotype x environment interactions, emphasizing their substantial influence on the mean sum of squares for key economic traits in Ashwagandha. The analysis of root length across all crosses indicated high stability and predictability, except two crosses. Crosses like UWS-98 x UWS-10, UWS-100 x UWS-10, UWS-120 x UWS-10, GP-49 x UWS-10, UWS-13 x UWS-23, UWS-18 x UWS-23, UWS-92 x UWS-23, UWS-104 x UWS-23, UWS-131 x UWS-23, UWS-18 x UWS-60, UWS-92 x UWS-60, UWS-98 x UWS-60, and GP-51 x UWS-60 displayed stable performance with regression coefficients less

Table 1. Analysis of variance Eberhart and Russel (1966)

S.N.	Characters	Genotype	E+(G x E)	E (L)	G x E (L)	Pool dev.	Pool Error
1.	Root length (cm)	26.87**	7.54**	1183.45**	0.78*	0.54**	2.24
2.	Number secondary and tertiary root per plant	0.29**	0.16**	22.73**	0.03	0.02	0.05
3.	Dry root yield (g/plant)	1.06**	0.25**	10.44**	0.33**	0.04	0.53
4.	Total alkaloid (%) content	0.015**	0.003**	0.006	0.005**	0.002	0.014

Table 2. Stability parameters for Root Length, Number of secondary and tertiary roots, Dry root yield & Total Alkaloid content

S.		Root Length (cm)			Number of sec. & ter. Roots			Dry root yield			Total Alkaloid content		
No.	Crosses	b 0	b 1	S^2d^i	b 0	b 1	S^2d^i	b 0	b 1	S^2d^i	b 0	b 1	S^2d^i
1	UWS-13 x UWS-10	22.44	0.99**	-2.170	2.99	1.00**	-0.044	2.66	0.26++	-0.522	0.39	-8.96	-0.00
2	UWS-18 x UWS-10	18.73	1.03**	-2.217	2.65	1.03**	-0.034	3.30	2.21**++	-0.519	0.31	-5.11	-0.01
3	UWS-79 x UWS-10	22.36	1.06**+	-2.233	3.13	1.00**	-0.044	2.75	3.87**+	-0.372	0.39	7.43**++	-0.01
4	UWS-89 x UWS-10	25.03	1.25**+	-2.071	3.12	1.00**	-0.044	2.96	-1.06++	-0.493	0.41	12.25**++	-0.013
5	UWS-92 x UWS-10	17.79	1.21**	-1.955	3.38	1.34**++	-0.046	4.19	0.56	-0.417	0.44	6.11*+	-0.013
6	UWS-98 x UWS-10	24.82	0.86**	-1.873	3.52	1.02**	-0.040	2.77	-0.80++	-0.500	0.50	5.27**++	-0.013
7	UWS-100 x UWS-10	22.94	0.96**	-1.714	4.11	1.00**	-0.044	3.46	-0.80++	-0.500	0.45	-10.28+	-0.012
8	UWS-104 x UWS-10	21.85	1.24**++	-2.232	2.95	1.00**	-0.044	3.20	0.72**++	-0.526	0.43	-19.94+	-0.006
9	UWS-106 x UWS-10	27.35	1.04**	-1.678	3.58	1.27**	-0.022	2.95	-1.64++	-0.472	0.34	-6.98++	-0.013
10	UWS-120 x UWS-10	24.09	0.93**	-2.060	3.40	1.33**	-0.013	3.50	2.24**++	-0.519	0.35	-3.20	-0.013
11	UWS-122 x UWS-10	21.62	0.89**	-1.969	2.50	1.00**	-0.044	2.90	0.52**++	-0.524	0.52	12.09**++	-0.013
12	UWS-129 x UWS-10	25.68	1.17**++	-2.199	3.34	2.06*	0.157*	3.35	1.58**	-0.500	0.37	9.57*	-0.012
13	UWS-131 x UWS-10	17.14	1.33**	-1.838	2.84	1.03**	-0.031	2.10	1.23**++	-0.527	0.41	-2.83	-0.013
14	UWS-132 x UWS-10	24.34	1.06**	-1.803	3.53	1.00**	-0.044	3.03	-3.84++	-0.352	0.27	11.56**++	-0.013
15	UWS-134 x UWS-10	21.89	1.31**+	-1.915	3.21	1.29**++	-0.045	2.03	1.95	-0.378	0.30	2.35**++	-0.014
16	UWS-135 x UWS-10	23.90	1.03**	-2.217	3.49	1.00**	-0.044	2.93	1.63**++	-0.526	0.45	10.05	-0.001
17	UWS-140 x UWS-10	25.48	1.22**+	-2.119	3.21	1.28**	-0.042	2.10	1.15**	-0.508	0.53	10.90	-0.007
18	GP-49 x UWS-10	23.19	0.96**	-2.127	3.21	1.14**	-0.034	2.04	-0.63++	-0.505	0.45	-12.40	-0.009
19	GP-50 x UWS-10	21.37	0.78**	-1.554	2.98	1.00**	-0.044	3.32	5.44**++	-0.527	0.38	-15.02+	-0.011
20	GP-51 x UWS-10	22.90	1.25**+	-2.075	2.91	1.00**	-0.044	3.31	0.72**++	-0.526	0.37	-12.48++	-0.012
21	UWS-13 x UWS-23	26.88	0.99**	-2.076	2.58	1.00**	-0.044	3.58	0.88**	-0.527	0.32	-1.80	-0.013
22	UWS-18 x UWS-23	25.73	0.94**	-2.086	3.26	1.27**	-0.022	3.40	2.32**	-0.454	0.49	4.57*	-0.013
23	UWS-79 x UWS-23	16.94	1.24**+	-2.084	3.56	1.00**	-0.044	2.67	1.42**++	-0.527	0.35	11.61*+	-0.012
24	UWS-89 x UWS-23	20.10	0.94**	-2.086	3.06	1.00**	-0.044	3.25	1.92**++	-0.523	0.46	3.62**+	-0.013
25	UWS-92 x UWS-23	26.10	0.69	1.703	2.91	1.00**	-0.044	2.24	-1.36++	-0.483	0.41	3.55	-0.013
26	UWS-98 x UWS-23	17.30	0.98**	-0.995	3.05	0.13	0.015	3.19	-0.52++	-0.514	0.40	0.76	-0.013
27	UWS-100 x UWS-23	20.81	1.03**	-2.212	2.48	1.00**	-0.044	2.89	1.32**++	-0.527	0.43	-10.45++	-0.013
28	UWS-104 x UWS-23	25.26	0.20	3.403	3.03	1.00**	-0.044	3.32	2.48**++	-0.515	0.42	3.76	-0.013
29	UWS-106 x UWS-23	20.78	1.06**++	-2.236	3.14	1.00**	-0.044	3.12	1.04**	-0.527	0.44	-6.58++	-0.013
30	UWS-120 x UWS-23	21.40	1.03**	-2.217	3.15	1.03**	-0.035	2.64	-1.67++	-0.471	0.38	1.91	-0.013
31	UWS-122 x UWS-23	24.50	1.10**++	-2.241	3.45	1.36**	-0.009	3.42	0.89**	-0.527	0.51	6.66**++	-0.013
32	UWS-129 x UWS-23	25.93	1.19**++	-2.169	3.07	1.03**	-0.034	3.36	3.23**++	-0.519	0.35	5.95	-0.013
33	UWS-131 x UWS-23	23.19	0.96**	-2.040	3.33	0.37	0.076	3.06	-1.01++	-0.494	0.46	1.51	-0.013
34	UWS-132 x UWS-23	21.37	1.12**++	-2.235	2.80	1.06	0.034	2.54	-0.50++	-0.508	0.36	3.22**++	-0.014
35	UWS-134 x UWS-23	19.68	1.27**++	-2.154	3.08	1.04**	-0.022	2.64	1.93	-0.342	0.45	6.75	-0.012
36	UWS-135 x UWS-23	20.08	1.03**	-2.045	2.80	1.04**	-0.014	3.36	2.96**++	-0.465	0.34	0.39	-0.013
37	UWS-140 x UWS-23	17.89	1.44**++	-2.134	3.44	1.22**	-0.007	3.48	-1.34++	-0.452	0.44	8.24	-0.005
38	GP-49 x UWS-23	22.39	1.25**	-1.894	2.60	1.04**	-0.024	2.24	1.79**+	-0.514	0.40	0.42	-0.013
39	GP-50 x UWS-23	25.24	1.08**++	-2.241	2.97	1.03**	-0.035	3.02	-0.24	-0.418	0.47	-1.66	-0.013
40	GP-51 x UWS-23	21.76	0.71**++	-2.119	3.02	1.04**	-0.018	3.30	-0.68++	-0.503	0.34	-9.76	-0.011
41	UWS-13 x UWS-60	19.47	1.06**	-1.893	2.88	1.01**	-0.048	3.72	3.62**++	-0.523	0.59	-8.41++	-0.013
42	UWS-18 x UWS-60	26.34	0.60**+	-1.855	2.83	1.01**	-0.048	2.79	-1.56++	-0.475	0.43	1.53*	-0.013
43	UWS-79 x UWS-60	24.02	1.21*	2.260	3.43	0.05++	-0.048	2.32	-0.20+	-0.498	0.46	-16.56++	-0.012
44	UWS-89 x UWS-60	21.15	1.04**	-2.219	3.08	1.01**	-0.048	3.03	3.13**+	-0.384	0.46	-2.32	-0.013
45	UWS-92 x UWS-60	25.35	0.75	3.990	3.33	0.50	0.012	2.99	2.34**++	-0.519	0.49	13.57**++	-0.013
46	UWS-98 x UWS-60	23.09	0.84*	-0.314	2.97	1.02**	-0.047	2.54	-0.55	-0.317	0.42	-2.83	-0.009
47	UWS-100 x UWS-60	22.20	1.04**	-1.591	2.92	2.57**	0.151*	3.43	2.09**++	-0.508	0.55	13.93	0.001
48	UWS-104 x UWS-60	23.04	1.07**	-1.933	2.95	1.44**++	-0.042	4.12	0.02	-0.444	0.39	19.52**++	-0.011
49	UWS-106 x UWS-60	18.36	0.52	0.074	2.82	1.11*	0.008	2.75	1.10**++	-0.527	0.35	3.02**+	-0.013
50	UWS-120 x UWS-60	20.66	1.04**	-2.222	2.88	0.89**	-0.040	3.27	2.10**++	-0.525	0.37	0.19	-0.013
51	UWS-122 x UWS-60	22.20	1.21**+	-2.144	3.02	1.19**+	-0.046	2.93	0.14++	-0.520	0.39	-6.39	-0.011
52	UWS-129 x UWS-60	25.83	1.34**	-1.816	2.74	1.25**++	-0.048	2.18	0.38	-0.302	0.30	1.61	-0.013
53	UWS-131 x UWS-60	24.43	1.12**	-1.775	2.91	1.03**	-0.028	2.09	0.00++	-0.518	0.46	-0.63	-0.013
54	UWS-132 x UWS-60	23.65	1.17**	-1.199	3.41	0.56	-0.020	2.34	1.13**	-0.517	0.30	3.35	-0.013

Table 2. *Continued ...*

S. No.	Crosses	Root Length (cm)			Number of sec. & ter. Roots			Dry root yield			Total Alkaloid content		
		b 0	b 1	S^2d^i	b 0	b 1	S^2d^i	b 0	b 1	S^2d^i	b 0	b 1	S^2d^i
55	UWS-134 x UWS-60	18.39	0.36**++	-2.240	2.85	1.05*	0.006	2.00	0.36	-0.463	0.40	5.81*+	-0.013
56	UWS-135 x UWS-60	23.51	1.08**++	-2.241	3.12	0.99**	-0.033	2.06	1.91	-0.407	0.36	-2.32	-0.012
57	UWS-140 x UWS-60	26.95	1.18**	-1.039	3.35	1.00**	-0.044	2.56	1.56	-0.407	0.31	2.25	-0.011
58	GP-49 x UWS-60	22.06	1.22**+	-2.130	2.55	0.85**++	-0.048	2.55	1.39**++	-0.527	0.36	7.78**++	-0.013
59	GP-50 x UWS-60	22.91	1.24**+	-2.084	2.65	0.62*	-0.025	2.07	1.14**	-0.507	0.30	3.14	-0.013
60	GP-51 x UWS-60	24.12	0.98**	-2.161	2.90	1.01**++	-0.048	3.28	0.93**	-0.527	0.44	-4.81++	-0.013

than one (b^i<1) and mean values exceeding the average (22.60) across all crosses (Table 2). These findings highlight the suitability of specific crosses for stable root growth across varying environmental conditions. For number of secondary and tertiary roots detailed in Table 3 all crosses except two exhibited non-significant deviation from regression (S^2d_i), indicating their stability and predictability. Among crosses *viz*., UWS-135 x UWS-60, UWS-132 x UWS-60, UWS-92 x UWS-60, UWS-79 x UWS-60 and UWS-131 x UWS-2 displayed regression coefficients less than one (b^i<1) and mean values higher than the average mean of all crosses (3.07). This signifies their stability even under favorable environmental conditions. The assessment of dry root yield across various crosses (referenced in Table 2) like UWS-92 x UWS-10, UWS-100 x UWS-10, UWS-104 x UWS-10, GP-51 x UWS-10, UWS-13 x UWS-23, UWS-98 x UWS-23, UWS-122 x UWS-23, GP-50 x UWS-23, GP-51 x UWS-23, UWS-104 x UWS-60, UWS-122 x UWS-60, and GP-51 x UWS-60 displayed stability with regression coefficients less than one (b^i<1) and mean values higher than the average mean of all crosses (2.91). The findings highlight the economic importance of ashwagandha roots. Incorporating stable crosses into cultivation practices enhances resilience to adverse environments, thereby supporting the sustainability of ashwagandha production over time. Lal 2015, Kumar *et al*. (2020), Ahmed *et al.* (2024) and (Yadav *et al*., 2024) also reported adaptability and stability for root yield over the years. For total alkaloid content all crosses exhibited non-significant deviation from regression (S^2d_i), indicating their stability and predictability. Cross UWS-131 x UWS-60 showed regression coefficients less than one (b^i<1) and mean values higher than the average mean of all parents (0.41). This signifies their stability even under unfavorable environmental conditions. Consistency in the yield of dried roots and the percentage of total alkaloid content is essential for maintaining reliable production levels and meeting market demands. This stability contributes significantly to the sustainability and profitability of Ashwagandha cultivation practices, ensuring they remain economically viable and meet consumer needs effectively. These findings are similar with prior research conducted by Kumar *et al.* (2020).

In conclusion, several crosses consistently displayed stable and predictable performance across key traits essential for ashwagandha cultivation. Notably, crosses such as UWS-18 x UWS-10, UWS-104 x UWS-10, UWS-120 x UWS-10, UWS-131 x UWS-10, UWS-132 x UWS-10, UWS-100 x UWS-23, UWS-104 x UWS-23, UWS-106 x UWS-23, and GP-51 x UWS-23 demonstrated stability across root length, dry root yield and alkaloid content. These crosses consistently performed well, exhibiting resilience and dependable output under different environmental conditions. Their suitability across multiple critical traits highlights their potential to enhance ashwagandha production sustainability and economic viability.

Conflict of Interest-None

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