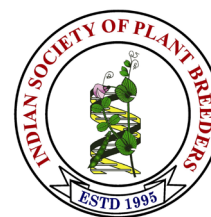


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Research Article

Evaluation of stability for root yield and its attributing traits in ashwagandha [*Withania somnifera* (L.) Dunal]

Deepak Meena^{1*}, Amit Dadheech¹, Hemlata Sharma¹, Amit Kumar¹, Preeti Bassar¹, Monika Shahani¹ and Ashish Meena²

¹Department of Genetics and Plant Breeding, RCA, MPUAT, Udaipur (Rajasthan), India

²Department of Agronomy, RCA, MPUAT, Udaipur, (Rajasthan), India

*E-Mail: deepakmeena152@gmail.com

Abstract

An experiment to evaluate the stability of Ashwagandha genotypes was conducted during the *Rabi* season of 2022-23 across three different environments. A significant genotype x environment (G x E) interaction was observed, indicating a strong environmental influence for all the traits studied. Based on the stability parameters, the crosses $L_{13} \times T_3$, $L_{14} \times T_3$, $L_{15} \times T_3$, $L_{13} \times T_1$, $L_3 \times T_2$, $L_6 \times T_2$, $L_{12} \times T_2$, $L_{13} \times T_2$ and $L_{14} \times T_2$ showed non-significant deviations, b_i values less than unity ($b_i < 1$) and a mean higher than the general mean (2.75), indicating above average stability. The crosses $L_4 \times T_1$, $L_7 \times T_1$, $L_8 \times T_1$, $L_7 \times T_2$, $L_2 \times T_3$ and $L_{12} \times T_3$ showed a mean higher than the general mean (2.75) and b_i values more than unity ($b_i > 1$), indicating below average stability for dry root yield. Among the checks, JA-20 showed regression coefficient less than one ($b_i < 1$) and mean higher than the check mean (4.50) indicating above average stability for dry root yield. Therefore, the identified stable genotypes can be effectively utilized in breeding programs aimed at improving dry root yield and alkaloid content in ashwagandha.

Keywords: ashwagandha, dry root yield, total alkaloid content, stability

INTRODUCTION

India is home to a rich heritage of natural biodiversity, including a wide variety of medicinal plants used for various purposes. Many medicinal plants naturally thrive in the Western Ghats, while some are cultivated commercially based on their demand and economic value. Ashwagandha [*Withania somnifera* (L.) Dunal], a member of the *Solanaceae* family is native to regions such as North Africa, Western Asia, South Asia, Southern Europe, the Mediterranean, and the Canary Islands (Datta *et al.*, 2010). It is one of the most significant medicinal plant species, revered by ancient Indians for centuries. The name "Ashwagandha" refers to the distinct smell of its roots, which is said to resemble that of a horse (*ashwa* meaning horse). Commonly known as Indian ginseng or winter cherry, it is widely recognized for its diuretic, restorative, aphrodisiac and rejuvenating properties. These attributes make it a key ingredient in traditional Indian medicinal systems such as Ayurveda,

Siddha and Unani. Due to its aphrodisiac and restorative qualities, Ashwagandha roots are often compared to ginseng roots. In recent times, it has attracted significant interest from modern pharmacologists and chemists. The plant particularly its leaves and roots is known to be rich in various medicinal properties. Its chromosome number is $2n=48$ (Nigam and Kandalkar, 1995). *W.somnifera* exhibits at least five distinct morphological forms displaying a high degree of variability in growth habits and morphological characteristics across different regions of India and other parts of the world (Atel and Schwarting, 1962). The genus *Withania* (*Solanaceae*) consists of 76 species distributed widely in South Asia and the Eastern Mediterranean region (Kaul *et al.*, 2005). Among these, only two species *W.coagulans* and *W. somnifera* have been reported in India. Many traits in Ashwagandha, including root yield hold significant economic importance as they depend on several other characters. These traits

are often polygenically (quantitatively) inherited, making them highly susceptible to environmental fluctuations. The variability observed within a population tends to change with the environment due to genotype x environment interactions. Plant breeders are increasingly concerned about these interactions, as selections made under specific environmental conditions may not perform effectively in different environments. A cultivar must perform well across a wide range of environments to be successful at the commercial level. When genotypes are tested across numerous environments, they reveal specificity or performance in particular environments. Some genotypes demonstrate better performance across varied environments. Quantitative traits like dry root yield are greatly affected by environmental factors and any variation in the performance of a genotype across different environments is known as genotype x environment (G x E) interaction (Manuel *et al.*, 1997, Dwivedi *et al.*, 2020 and Philanim *et al.*, 2022). In Ashwagandha the total alkaloid content is a key economic trait that is highly influenced by environmental conditions (Li *et al.*, 2018 and Munaro *et al.*, 2011). To ensure consistent performance of promising genotypes across varying environments, it is important to conduct stability analysis.

MATERIALS AND METHODS

The current study was conducted during the *Rabi* season of 2022-23 across three distinct locations/environments in the southern region of Rajasthan: (E₁) Instructional Farm, Rajasthan College of Agriculture, Udaipur, (E₂) Krishi Vigyan Kendra, Chittorgarh and (E₃) Agricultural Research Station, Banswara. At each location, the trial was arranged in three replications. The experimental material consisted of 16 lines *viz.*, UWS-16 (L₁), UWS-20 (L₂), UWS-21 (L₃), UWS-22 (L₄), UWS-35 (L₅), UWS-40 (L₆), UWS-46 (L₇), UWS-47 (L₈), UWS-57 (L₉), UWS-58 (L₁₀), UWS-65 (L₁₁), UWS-76 (L₁₂), UWS-80 (L₁₃), UWS-83 (L₁₄), UWS-84 (L₁₅) and UWS-85 (L₁₆); three testers *viz.*, UWS-10 (T₁), UWS-23 (T₂) and UWS-37 (T₃), obtained from AICRP-M&AP, Udaipur and their 48 F₁s along with three checks obtained from AICRP-

M&AP, Udaipur namely JA-20, JA-134 and RVA-100. The 48 F₁s experimental hybrids were generated through hybridization, involving 16 lines (females) and 3 testers (males) in a line x tester mating design (Kempthorne, 1957) during the *Rabi* season of 2021-22. Observations were recorded on following characters *viz.*, days to 50 % flowering and days to 75 % maturity (on plot basis), while for other remaining traits on plant basis by using 10 randomly selected plants *i.e.* number of berries per plant, number of secondary branches per plant, dry root yield per plant, number of secondary and tertiary roots per plant, test weight of seed and total alkaloid content (Misra, 1996). The pooled data from all three environments for the aforementioned characters were subjected to statistical analysis for stability, following the model proposed by Eberhart and Russell (1966).

RESULTS AND DISCUSSION

Analysis of variance based on phenotypic stability (Table 1) revealed that the mean squares for genotypes, environment and genotype x environment (G x E) interactions, including G x E (linear), were highly significant for all the characters studied. The genotypes or treatments showed considerable differences in stability making predictions for these traits challenging. The significance of the mean squares due to pooled deviation indicated that both linear and non-linear components played an important role in the total G x E interactions for these characters. The character-wise stability parameters are presented in Tables 2a and 2b.

Among the parents, 8 parent (including lines and testers) showed non-significant deviations from regression for days to 50% flowering, indicating their predictable response to the various environments for this trait. The parental lines L₁, L₃ and L₄ were found to express non-significant deviations, b_i values less than unity (b_i<1) and a mean lower than the general mean (94.59), indicating above average stability. The line L₁₅ (b_i>1) showed a mean lower than the general mean (94.59) and b_i values more than unity (b_i>1), indicating below average stability

Table 1. ANOVA for stability for different characters in ashwagandha (Eberhart and Russel, 1966 Model)

S.N.	Characters	Genotype [69]	E+(G x E) [140]	E (L) [1]	G x E (L) [69]	Pool dev. [70]	Pool Err [414]
1	Days to 50 % flowering	21.44**	21.67**	1288.16**	10.60**	14.49**	1.12
2	Days to 75 % maturity	109.47**	14.20**	1176.47**	5.64**	6.04**	1.05
3	Number of secondary branches per plant	14.97**	0.85**	80.62**	0.50**	0.04	0.09
4	Number secondary and tertiary root per plant	1.71**	0.27**	26.12**	0.11**	0.05**	0.02
5	Dry root yield (g)	1.94**	0.14**	13.65**	0.06**	0.04**	0.02
6	Number of berries per plant	3418.94**	236.59**	22420.85**	89.80**	64.36**	33.29
7	Test weight (g)	0.29**	0.04**	3.27**	0.02**	0.01**	0.00
8	Alkaloid content (%)	0.01**	0.00**	0.05**	0.00**	0.00**	0.00

*, ** Significant at 5% and 1% respectively

Table 2a: Stability parameters for days to 50 % flowering, days to 75 % maturity, number of secondary branches per plant and number of secondary and tertiary roots per plant

S.N.	Genotype	Days to 50 % flowering			Days to 75 % maturity			Number of secondary branches per plant			Number of secondary and tertiary roots per plant		
		μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i
1	T ₁	91.00	0.09	-0.38	160.00	1.32	-0.99	13.67	2.32	-0.05	4.75	1.48	0.03
2	T ₂	98.00	0.29	2.02	165.78	1.54	0.31	8.22	2.78*	0.03	4.27	0.73	-0.01
3	T ₃	99.33	0.68	-0.85	159.22	0.33	5.16*	8.91	1.64	-0.08	2.96	0.75	-0.01
4	L ₁	93.11	0.93	-1.04	153.44	0.69	-1.05	6.98	1.19	-0.03	5.01	1.59	0.17**
5	L ₂	94.11	0.18	7.46**	155.78	0.58	-1.05	8.01	1.51	-0.07	4.92	1.43	0.01
6	L ₃	91.33	0.83	-1.12	159.78	0.81	-1.04	5.21	0.52	-0.09	4.34	0.52	-0.02
7	L ₄	94.44	-0.16	-1.07	152.67	0.14	-0.48	9.33	0.75	-0.08	3.56	0.87	-0.00
8	L ₅	97.33	0.62	14.68**	163.00	0.41	14.15**	4.77	0.62	0.03	3.15	0.92	-0.02
9	L ₆	93.33	0.25	10.42**	162.00	-0.43	0.02	4.33	1.06	-0.06	2.45	0.81	-0.02
10	L ₇	94.11	0.60	49.47**	154.33	0.71	1.44	5.67	0.75	-0.08	3.19	0.78	-0.02
11	L ₈	96.44	0.25	9.83**	165.78	0.44	0.62	7.67	0.76	-0.08	4.04	3.89	0.17**
12	L ₉	95.00	1.25	34.91**	159.78	1.47	7.05**	12.78	1.76	-0.05	3.19	0.83	0.00
13	L ₁₀	98.00	0.57	55.75**	157.67	0.91	5.20*	8.33	1.52	-0.06	4.33	0.49	-0.00
14	L ₁₁	98.33	0.55	76.04**	164.22	0.91	-0.54	11.78	0.25	-0.09	5.06	0.87	0.07*
15	L ₁₂	97.00	1.63	0.61	158.56	0.95	0.27	7.80	1.37	-0.07	6.59	1.98	-0.02
16	L ₁₃	92.67	0.31	7.97**	162.89	1.09	-0.89	9.04	0.34	-0.09	3.50	0.79	0.00
17	L ₁₄	88.33	-0.06	4.82*	158.44	0.08	-0.63	5.11	1.76	-0.05	3.18	0.41	-0.02
18	L ₁₅	93.11	1.76	2.15	159.22	2.10	4.73*	8.78	0.25	-0.09	2.15	0.53	-0.02
19	L ₁₆	92.33	0.05	19.49**	161.78	0.44	-0.47	6.45	1.76	-0.05	3.58	0.57	-0.02
20	L ₁ x T ₁	99.11	0.53	1.25	165.56	1.73	13.51**	4.67	0.76	-0.08	3.86	1.18	0.08*
21	L ₂ x T ₁	94.00	1.55	2.62	159.11	1.70	10.72**	4.68	1.57	-0.05	3.02	0.82	-0.00
22	L ₃ x T ₁	98.67	0.33	45.50**	164.44	0.85	-0.74	6.45	2.52*	-0.01	3.34	2.15	0.28**
23	L ₄ x T ₁	94.56	1.95	-0.73	154.00	0.50	6.30**	8.67	0.40	-0.05	3.59	0.35	-0.02
24	L ₅ x T ₁	91.11	0.33	5.00*	167.11	0.53	1.61	7.78	1.41	-0.09	3.01	1.08	-0.02
25	L ₆ x T ₁	96.56	0.30	55.57**	164.67	0.73	0.61	7.44	0.65	-0.07	2.96	0.47	-0.02
26	L ₇ x T ₁	96.33	1.16	46.00**	147.89	0.09	24.23**	9.46	0.70	-0.07	3.75	0.49	-0.02
27	L ₈ x T ₁	98.67	0.42	34.48**	145.00	0.14	22.62**	4.90	0.26	-0.07	3.64	0.31	-0.02
28	L ₉ x T ₁	97.89	0.52	31.48**	158.89	1.34	13.42**	8.44	0.65	-0.07	2.66	0.68	-0.01
29	L ₁₀ x T ₁	100.78	0.33	5.00*	161.22	2.14	-1.04	9.00	0.40	-0.05	3.20	0.90	-0.00
30	L ₁₁ x T ₁	98.11	1.62	13.92**	163.00	0.51	10.88**	8.92	0.22	-0.05	3.81	0.76	0.06*
31	L ₁₂ x T ₁	98.22	0.25	62.25**	162.44	0.74	1.38	11.33	0.40	-0.05	3.25	1.05	0.03
32	L ₁₃ x T ₁	92.56	0.27	4.23*	156.67	0.89	0.53	10.45	1.36	0.07	3.12	0.51	-0.02
33	L ₁₄ x T ₁	96.67	1.94	-0.99	155.78	1.58	-0.51	8.26	0.69	-0.08	3.54	1.50	-0.02
34	L ₁₅ x T ₁	94.78	1.47	1.20	168.00	0.78	0.39	5.10	0.27	-0.09	3.12	0.24	0.03
35	L ₁₆ x T ₁	94.56	1.13	11.27**	159.44	1.15	-0.97	11.46	0.60	-0.01	3.67	1.32	-0.02
36	L ₁ x T ₂	95.11	0.83	20.85**	166.44	1.38	1.70	10.20	2.46*	-0.06	3.56	0.65	-0.01
37	L ₂ x T ₂	97.33	1.39	39.52**	161.22	1.20	5.22*	6.96	0.55	-0.09	3.14	1.13	0.02
38	L ₃ x T ₂	94.22	1.32	4.53*	162.00	1.62	15.58**	5.02	0.74	-0.09	3.56	0.65	-0.02
39	L ₄ x T ₂	95.11	1.07	28.04**	162.67	2.49	3.09*	6.37	1.07	-0.01	3.56	1.64	0.03
40	L ₅ x T ₂	98.44	0.85	11.36**	161.56	1.29	10.31**	7.89	0.91	-0.07	3.67	0.81	0.01
41	L ₆ x T ₂	98.89	0.34	24.32**	156.89	0.47	1.97	6.70	0.73	-0.09	3.71	1.58	-0.02
42	L ₇ x T ₂	97.44	1.12	36.29**	163.56	0.79	-0.61	6.89	0.55	0.08	3.23	1.19	0.16**
43	L ₈ x T ₂	91.44	2.00	-0.90	166.44	1.08	0.89	8.67	0.56	-0.09	3.91	1.51	0.06

S.N.	Genotype	Days to 50 % flowering			Days to 75 % maturity			Number of secondary branches per plant			Number of secondary and tertiary roots per plant		
		μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i
44	$L_9 \times T_2$	94.78	-0.11	17.64**	161.00	1.41	8.91**	5.60	0.77	-0.09	3.32	1.27	-0.01
45	$L_{10} \times T_2$	93.11	0.97	30.65**	147.89	0.50	0.22	6.22	0.91	-0.07	2.95	1.24	0.02
46	$L_{11} \times T_2$	97.89	1.25	-0.62	142.22	1.11	-0.88	5.44	1.41	-0.09	4.01	1.63	-0.02
47	$L_{12} \times T_2$	92.67	1.33	4.06*	157.00	0.41	-1.01	8.22	1.31	-0.05	3.21	0.34	-0.02
48	$L_{13} \times T_2$	94.11	1.21	23.12**	157.22	0.40	-1.05	4.93	0.47	-0.09	3.96	0.64	-0.02
49	$L_{14} \times T_2$	94.78	1.71	21.34**	158.67	1.09	3.69*	5.17	1.72	-0.08	3.56	1.11	0.00
50	$L_{15} \times T_2$	94.00	0.46	0.96	144.78	0.98	92.38**	5.03	0.36	-0.06	3.41	0.53	-0.02
51	$L_{16} \times T_2$	95.78	0.79	8.26**	156.56	0.88	-0.77	8.35	0.09	0.12	4.14	0.76	-0.01
52	$L_1 \times T_3$	96.89	1.75	1.24	151.33	1.30	8.34**	7.81	1.42	-0.09	3.51	1.15	0.00
53	$L_2 \times T_3$	94.00	1.51	11.54**	154.00	0.48	1.10	6.43	0.86	0.14	3.56	1.05	0.03
54	$L_3 \times T_3$	96.22	1.01	-0.71	154.78	1.61	1.34	9.10	2.18	-0.09	3.86	1.74	-0.02
55	$L_4 \times T_3$	98.89	0.35	-0.24	156.89	1.41	10.69**	11.47	0.24	-0.09	3.84	1.30	0.06*
56	$L_5 \times T_3$	100.00	0.28	4.35*	157.33	0.75	3.00*	8.11	1.41	-0.09	3.47	0.68	0.29**
57	$L_6 \times T_3$	95.11	1.62	13.92**	152.00	1.39	2.61	7.43	2.53*	0.01	4.04	0.21	-0.01
58	$L_7 \times T_3$	94.00	2.69	-0.47	154.33	1.67	1.68	8.83	0.57	-0.09	4.13	1.10	0.11*
59	$L_8 \times T_3$	93.67	0.04	1.52	158.00	1.67	4.99*	8.25	0.22	-0.05	3.35	1.63	0.07*
60	$L_9 \times T_3$	93.89	2.49	4.80*	158.33	1.44	2.06	8.89	1.64	-0.06	3.61	1.34	0.01
61	$L_{10} \times T_3$	97.33	1.25	-0.17	159.11	1.56	3.49*	7.56	0.91	-0.07	3.54	1.18	0.01
62	$L_{11} \times T_3$	95.78	2.55	2.25	148.56	1.48	25.68**	11.73	0.89	-0.08	3.84	0.96	0.24**
63	$L_{12} \times T_3$	94.00	2.48	3.56*	150.11	2.34	6.98**	9.54	0.57	-0.06	3.89	0.89	-0.01
64	$L_{13} \times T_3$	96.89	0.40	1.29	140.33	0.41	-1.01	9.26	0.40	-0.05	3.60	0.54	-0.02
65	$L_{14} \times T_3$	96.78	1.11	2.91	149.56	1.22	1.82	8.36	0.81	0.29*	3.65	0.98	-0.01
66	$L_{15} \times T_3$	89.89	2.49	4.80*	159.22	1.27	-1.04	8.98	0.69	-0.09	5.04	1.18	0.01
67	$L_{16} \times T_3$	93.33	1.25	-0.17	165.56	1.57	4.01*	8.50	0.73	-0.09	5.34	0.96	0.24**
68	Check 1	91.78	2.55	2.25	159.00	0.36	0.94	12.66	0.95	-0.02	5.39	0.89	-0.01
69	Check 2	90.00	2.48	3.56*	153.56	0.54	-0.57	10.00	2.27	-0.02	5.10	0.54	-0.02
70	Check 3	92.89	0.40	1.29	155.33	0.53	-1.03	12.41	0.29	-0.09	5.15	0.98	-0.01

*, ** and +, ++ Significantly deviating from 0 and 1 at 5% and 1% respectively.

(Table 2a). Nineteen crosses out of the 48 exhibited non-significant deviations from regression, indicating their predictable response to the various environments for days to 50 % flowering. The crosses $L_4 \times T_1$, $L_8 \times T_2$, $L_9 \times T_2$, $L_{10} \times T_2$, $L_{15} \times T_2$, $L_7 \times T_3$ and $L_{16} \times T_3$ showed non-significant deviations, b_i values less than unity ($b_i < 1$) and a mean lower than the general mean (95.72), indicating above average stability. The crosses $L_2 \times T_1$, $L_{15} \times T_1$ and $L_8 \times T_3$ showed a mean lower than the general mean (95.72) and b_i values more than unity ($b_i > 1$), indicating below average stability. Above-average stable genotypes (L_1 , L_3 , L_4 and crosses like $L_4 \times T_1$) are valuable for cultivation in variable or stress-prone environments, ensuring reliable flowering timelines. Below-average stable genotypes (L_{15} and its crosses) may require targeted environmental conditions or further genetic improvement to enhance adaptability. These findings align with previous studies by Ahmed and Dubey (2024) and Bassar *et al.* (2025), reinforcing the reliability of regression-based stability parameters in medicinal plant breeding.

With reference to days to 75% maturity, 11 parents expressed non-significant deviations from regression, indicating their predictable response to the various environments. The lines namely, L_1 , L_2 , L_7 , L_{12} and L_{14} expressed b_i values less than unity ($b_i < 1$) and a mean lower than the general mean (159.70) indicating above average stability (Table 2a). A total of 22 crosses, exhibited non-significant deviations from regression, indicating their predictable response to the various environments for days to 75% maturity. The crosses $L_{13} \times T_1$, $L_{10} \times T_2$, $L_{12} \times T_2$, $L_{13} \times T_2$, $L_{16} \times T_2$, $L_2 \times T_3$, $L_{13} \times T_3$ showed a mean less than the general mean (157.27) and regression coefficient less than unity ($b_i < 1$) which highlights their stability, particularly for unfavorable conditions (Table 2a). The crosses viz., $L_{14} \times T_1$, $L_6 \times T_2$, $L_{11} \times T_2$, $L_{14} \times T_2$, $L_3 \times T_3$, $L_6 \times T_3$, $L_7 \times T_3$, $L_{14} \times T_3$ showed a mean lower than the general mean (157.27) and regression coefficient more than unity ($b_i > 1$), which highlights their stability, particularly for favorable conditions. Among the checks, JA-20 and JA-134 showed regression coefficient lower

than one ($b_i < 1$) and mean lower than the check mean (155.96). Genotypes with $b_i < 1$ and lower mean values are particularly valuable for stress-resilient breeding, offering stable maturity timelines under suboptimal conditions.

Genotypes with $b_i > 1$ and lower mean values may be better suited for high-input systems, where favorable conditions can be leveraged for enhanced performance. The use of regression-based stability parameters provides a nuanced understanding of genotype $\times$ environment interactions, allowing breeders to tailor selections based on specific agro-climatic needs. These findings complement earlier observations on flowering stability and align with the broader goal of developing Ashwagandha cultivars with reliable maturity profiles. The inclusion of checks like JA-20 and JA-134 further validates the analytical framework and supports their continued use in breeding programs. Similar results were also reported by Sangwan *et al.* (2013) and Lal (2015) supporting the consistency of these stability patterns across different studies and environments.

All the parents showed non-significant deviations from regression for the trait number of secondary branches per plant, indicating their predictable response to the various environments (**Table 2a**). The parents *viz.*, L_4 , L_{10} , L_{11} , L_{13} and L_{15} expressed b_i values less than unity ($b_i < 1$) and a mean higher than the parental mean (8.04) indicating above average stability. The parents namely T_1 , T_3 and L_{10} showed b_i values more than unity ($b_i > 1$) and a mean higher than the parental mean (8.04) indicating below average stability for number of secondary branches per plant. All the crosses showed non-significant deviations from regression except one cross, indicating their predictable response to the various environments for number of secondary branches per plant.

Twenty crosses showed regression coefficient less than unity ($b_i < 1$) and a mean higher than the parental mean (7.81) indicating above average stability. In case of the crosses, $L_5 \times T_1$, $L_{13} \times T_1$, $L_{12} \times T_2$, $L_3 \times T_3$, $L_5 \times T_3$ and $L_9 \times T_3$ the regression coefficient was observed to be more than unity ($b_i > 1$) and the mean was higher than the parental mean (7.81) indicating below average stability for the trait. Among the checks, JA-134 and RVA-100 showed regression coefficient lower than one ($b_i < 1$) and mean higher than the check (11.69) indicating above average stability for number of secondary branches per plant.

The trait number of secondary branches per plant is a key determinant of biomass and overall plant architecture in Ashwagandha. Above-average stable genotypes are valuable for broad adaptability, ensuring consistent branching even under suboptimal conditions. Below-average stable genotypes may be leveraged in high-input systems, where favorable environments can maximize branching potential. The predominance of non-significant regression deviations across genotypes highlights the genetic stability of this trait, making it a reliable selection

criterion in breeding programs (Eberhart and Russell 1966 and Kumar *et al.*, 2020).

With respect to number of secondary and tertiary roots per plant, all the parents showed non-significant deviation from regression except four, indicating their predictable response to the various environments for number of secondary and tertiary roots per plant (**Table 2a**). This trait's stability enhances its reliability for selection in breeding programs (Eberhart and Russell 1966). Three parents *viz.*, T_2 , L_3 and L_{10} expressed b_i values less than unity ($b_i < 1$) and a mean higher than the parental mean (3.91) indicating above average stability, suggested that these genotypes are likely to maintain superior root development under diverse conditions. The parents T_1 , L_2 and L_{12} expressed b_i values more than unity ($b_i > 1$) and a mean higher than the parental mean (3.91) indicating below average stability suggesting their performance may be more environment-dependent and better suited to favorable settings for number of secondary and tertiary roots per plant. Among the crosses, except 10, all showed non-significant deviation from regression for number of secondary and tertiary roots per plant. The crosses *viz.*, $L_7 \times T_1$, $L_8 \times T_1$, $L_5 \times T_2$, $L_{13} \times T_2$, $L_{16} \times T_2$, $L_6 \times T_3$, $L_{12} \times T_3$ and $L_{14} \times T_3$ showed non-significant deviations, b_i values less than unity ($b_i < 1$) and a mean higher than the general mean (3.60), indicating above average stability whereas the crosses *viz.*, $L_{16} \times T_1$, $L_6 \times T_2$, $L_8 \times T_2$, $L_{11} \times T_2$, $L_3 \times T_3$, $L_9 \times T_3$ and $L_{15} \times T_3$ showed a mean higher than the general mean (3.60) and b_i values more than unity ($b_i > 1$), indicating below average stability for number of secondary and tertiary roots per plant. Among the checks, JA-20 showed regression coefficient less than one ($b_i < 1$) and mean higher than the check (5.21) indicating above average stability for number of secondary and tertiary roots per plant. Most crosses also showed non-significant regression deviations, reinforcing the trait's genetic consistency. Crosses such as $L_7 \times T_1$, $L_8 \times T_1$, $L_5 \times T_2$ and others with $b_i < 1$ and mean values above the general mean (3.60) are promising for broad adaptability. Conversely, crosses like $L_{16} \times T_1$, $L_6 \times T_2$ and $L_3 \times T_3$, which exhibited $b_i > 1$ and higher-than-average means, may be better suited for targeted environments where their potential can be fully realized. Similar findings were also reported by Sangwan *et al.* (2013) and Lal (2015).

Except four, all the parents showed non-significant deviation from regression indicating their predictable response to the various environments for dry root yield. The parents L_{11} , L_{12} , L_2 , L_3 and L_6 expressed b_i values less than unity ($b_i < 1$) and a mean higher than the parental mean (2.27) indicating above average stability (**Table 2b**). Such genotypes are particularly valuable in breeding programs aimed at developing cultivars with reliable performance under variable conditions. The parents T_3 and L_8 expressed b_i values more than unity ($b_i > 1$) and a mean higher than the parental mean (2.27) indicating below average stability for dry root yield. These genotypes may be more responsive to favorable

Table 2b: Stability parameters for dry root yield (g) and number of berries per plant, test weight (g) and alkaloid content (%)

S.N.	Genotype	Dry root yield (g)			Number of berries per plant			Test weight (g)			Alkaloid content (%)		
		μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i
1	T ₁	1.53	0.93	-0.02	78.06	1.26	17.67	3.01	1.77	0.00	0.36	0.46	-0.00
2	T ₂	2.90	2.28	0.05*	176.48	0.57	-32.20	3.26	0.28	-0.00	0.47	0.36	-0.00
3	T ₃	2.54	1.64	-0.02	135.19	1.01	-27.14	2.96	1.14	-0.00	0.38	0.45	-0.00
4	L ₁	1.26	0.64	-0.01	96.99	0.45	-31.97	2.77	2.35	-0.00	0.36	0.37	-0.00
5	L ₂	2.46	0.75	-0.01	148.41	0.68	-17.90	3.01	0.53	0.00	0.39	1.19	-0.00
6	L ₃	3.43	0.62	-0.00	186.22	0.89	-32.32	3.03	1.34	0.01	0.42	1.11	0.00
7	L ₄	2.39	1.24	0.02	122.21	0.77	-28.87	3.05	0.33	-0.00	0.30	1.31	0.00*
8	L ₅	2.06	0.38	-0.01	86.02	0.60	-33.04	2.46	0.97	0.00	0.36	0.74	0.00
9	L ₆	2.38	0.82	-0.01	85.94	0.81	-33.08	3.01	1.56	0.02*	0.37	0.28	-0.00
10	L ₇	1.70	0.54	-0.01	77.84	0.53	-32.55	2.94	1.48	-0.00	0.36	1.53	0.00*
11	L ₈	3.63	1.51	-0.02	170.72	0.44	-28.39	3.14	0.49	-0.00	0.46	0.28	-0.00
12	L ₉	1.30	0.46	-0.02	78.12	0.95	-33.19	2.72	1.23	0.00	0.36	0.18	-0.00
13	L ₁₀	1.74	1.41	0.05*	82.63	0.37	-33.03	2.75	1.41	-0.00	0.34	0.17	0.00
14	L ₁₁	3.16	0.58	-0.01	100.68	0.70	-20.92	2.95	0.49	0.00	0.35	0.46	-0.00
15	L ₁₂	3.21	0.80	-0.01	135.20	4.09	2143.58**	2.81	2.35	0.02*	0.42	0.37	-0.00
16	L ₁₃	2.07	1.53	0.05*	83.09	0.92	-1.85	3.06	1.67	0.02*	0.29	0.63	0.00
17	L ₁₄	1.21	0.52	-0.02	80.51	0.47	-33.24	2.92	1.51	0.02**	0.30	0.37	-0.00
18	L ₁₅	1.99	1.87	0.82**	87.99	0.25	-28.31	2.60	1.74	0.00	0.28	0.36	-0.00
19	L ₁₆	2.24	0.70	-0.01	101.93	0.67	59.84	2.63	1.12	-0.00	0.32	0.65	0.00
20	L ₁ x T ₁	1.96	0.54	-0.02	94.39	1.16	22.56	2.66	0.40	-0.00	0.31	0.54	-0.00
21	L ₂ x T ₁	1.98	0.63	-0.01	111.65	0.72	-21.29	2.91	0.11	0.21**	0.38	0.46	-0.00
22	L ₃ x T ₁	2.02	1.05	-0.00	80.71	0.56	-31.99	3.05	1.52	0.05**	0.38	0.80	0.00
23	L ₄ x T ₁	3.28	1.14	0.01	155.95	0.82	-29.50	3.19	0.91	0.00	0.39	0.28	-0.00
24	L ₅ x T ₁	1.87	1.10	-0.01	81.51	0.07	25.82	3.08	0.86	-0.00	0.35	0.37	-0.00
25	L ₆ x T ₁	1.97	1.19	-0.01	89.32	0.89	-31.01	2.54	1.69	-0.00	0.40	0.72	0.00
26	L ₇ x T ₁	3.24	1.02	-0.00	94.83	1.13	-1.58	2.64	1.44	0.02*	0.36	1.28	-0.00
27	L ₈ x T ₁	3.12	1.06	0.16**	153.51	1.30	-30.38	2.57	0.66	-0.00	0.43	1.08	0.00
28	L ₉ x T ₁	1.77	0.79	-0.00	79.98	1.61	40.95	2.94	1.88	0.02*	0.38	0.63	-0.00
29	L ₁₀ x T ₁	1.98	1.88	-0.01	83.13	0.86	-30.73	2.96	1.87	0.00	0.34	3.84	0.00**
30	L ₁₁ x T ₁	2.42	1.24	0.00	116.51	0.97	-26.96	3.06	0.65	-0.00	0.36	2.29	-0.00
31	L ₁₂ x T ₁	2.16	2.63	0.17**	87.35	0.91	-31.47	2.48	0.86	-0.00	0.34	1.75	0.00
32	L ₁₃ x T ₁	3.13	0.80	-0.02	148.38	1.25	37.18	2.73	0.67	-0.00	0.41	2.82	0.00
33	L ₁₄ x T ₁	2.69	1.58	0.00	100.47	0.66	-26.60	2.60	0.61	-0.00	0.35	0.28	-0.00
34	L ₁₅ x T ₁	2.27	1.04	0.00	121.47	1.38	-30.75	2.73	0.91	-0.00	0.40	2.58	0.00**
35	L ₁₆ x T ₁	2.24	0.75	0.00	107.90	1.20	-12.87	2.50	0.37	-0.00	0.35	1.18	-0.00
36	L ₁ x T ₂	2.36	2.19	0.10**	94.14	1.31	-33.11	3.03	0.86	-0.00	0.33	0.65	-0.00
37	L ₂ x T ₂	2.30	0.73	-0.01	110.67	0.48	-19.52	2.87	0.36	0.00	0.34	0.75	0.00
38	L ₃ x T ₂	2.95	0.87	-0.00	144.05	0.89	-27.80	2.95	1.30	0.00	0.46	1.73	-0.00
39	L ₄ x T ₂	2.01	0.43	-0.01	101.04	0.65	-18.49	2.72	0.75	-0.00	0.32	2.03	0.00
40	L ₅ x T ₂	2.68	1.08	0.00	93.38	0.96	20.83	2.60	0.41	-0.00	0.34	2.02	-0.00

S.N.	Genotype	Dry root yield (g)			Number of berries per plant			Test weight (g)			Alkaloid content (%)		
		μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i	μ_i	b_i	S^2d_i
41	$L_6 \times T_2$	2.89	0.98	-0.01	118.37	1.62	-23.99	2.82	0.91	0.00	0.35	2.77	0.00*
42	$L_7 \times T_2$	3.13	1.82	0.04	156.14	1.41	-19.11	2.86	0.58	-0.00	0.43	1.39	0.00
43	$L_8 \times T_2$	1.97	0.84	-0.02	94.49	1.07	-21.36	2.90	1.45	0.00	0.44	0.80	0.00*
44	$L_9 \times T_2$	2.37	1.74	0.02	91.33	1.02	-27.30	3.00	0.76	-0.00	0.37	0.74	0.00
45	$L_{10} \times T_2$	2.12	1.05	0.02	119.12	1.16	-3.87	2.99	1.87	0.02*	0.32	0.26	0.00
46	$L_{11} \times T_2$	2.68	1.66	-0.00	98.90	1.58	-14.82	3.09	-0.32	-0.00	0.35	2.40	0.00*
47	$L_{12} \times T_2$	3.71	0.41	-0.01	180.17	0.83	-13.55	2.64	0.59	0.00	0.40	1.00	-0.00
48	$L_{13} \times T_2$	3.17	0.29	-0.01	135.16	1.40	68.37	2.38	1.00	-0.00	0.41	2.28	-0.00
49	$L_{14} \times T_2$	3.21	0.41	-0.02	147.94	1.18	-30.25	2.48	2.61	0.07**	0.41	0.98	0.00
50	$L_{15} \times T_2$	2.74	0.88	0.01	119.18	0.76	364.85**	2.82	1.03	0.00	0.34	0.55	-0.00
51	$L_{16} \times T_2$	2.71	0.82	0.00	99.78	0.70	76.75	2.85	1.34	-0.00	0.37	0.79	0.00**
52	$L_1 \times T_3$	2.70	1.76	0.14**	160.18	0.90	179.54*	3.11	-0.07	-0.00	0.41	0.02	0.00
53	$L_2 \times T_3$	3.34	1.60	-0.01	185.85	0.82	39.37	2.94	1.78	-0.00	0.49	0.74	0.00
54	$L_3 \times T_3$	2.75	0.74	-0.02	122.74	0.63	13.31	3.09	0.98	-0.00	0.41	0.55	-0.00
55	$L_4 \times T_3$	2.35	0.95	-0.02	121.19	1.42	-14.48	2.70	2.16	0.00	0.43	0.34	0.00*
56	$L_5 \times T_3$	2.71	0.50	-0.02	108.33	1.79	29.51	2.54	1.37	-0.00	0.47	1.28	-0.00
57	$L_6 \times T_3$	3.38	1.33	0.15**	179.20	1.03	-9.04	2.72	1.47	-0.00	0.48	0.74	-0.00
58	$L_7 \times T_3$	2.27	0.55	-0.02	90.65	1.04	-1.17	2.91	1.43	0.00	0.37	0.54	0.00
59	$L_8 \times T_3$	2.21	0.63	0.01	111.20	1.49	-31.56	2.83	0.62	-0.00	0.34	0.90	0.00
60	$L_9 \times T_3$	2.93	0.43	-0.02	128.43	1.02	28.94	2.93	0.28	-0.00	0.38	0.73	-0.00
61	$L_{10} \times T_3$	2.71	1.12	-0.00	96.06	1.61	91.31	2.76	0.53	-0.00	0.45	1.30	0.00*
62	$L_{11} \times T_3$	3.74	1.56	0.12**	187.45	0.95	-32.60	2.79	0.41	-0.00	0.46	1.10	-0.00
63	$L_{12} \times T_3$	3.58	1.01	-0.01	127.40	1.51	39.06	2.60	0.83	0.00	0.43	0.65	0.00
64	$L_{13} \times T_3$	3.82	0.26	-0.02	165.85	0.53	-30.38	2.74	0.28	-0.00	0.38	1.27	0.00
65	$L_{14} \times T_3$	3.12	0.23	-0.02	165.19	0.82	-33.18	2.86	0.59	-0.00	0.35	1.10	-0.00
66	$L_{15} \times T_3$	4.11	0.95	-0.02	96.06	1.61	91.31	3.76	0.53	-0.00	0.48	1.30	0.00*
67	$L_{16} \times T_3$	5.04	1.56	0.12**	187.45	0.95	-32.60	3.79	0.41	-0.00	0.49	1.10	-0.00
68	Check 1	4.68	0.48	-0.01	127.40	1.51	39.06	3.60	0.83	0.00	0.46	0.65	0.00
69	Check 2	4.39	0.29	-0.02	165.85	0.53	-30.38	3.74	0.28	-0.00	0.41	1.27	0.00
70	Check 3	4.42	0.23	-0.02	165.19	0.82	-33.18	3.86	0.59	-0.00	0.38	1.10	-0.00

*, ** and +, ++ Significantly deviating from 0 and 1 at 5% and 1% respectively.

environments but less predictable under stress or suboptimal conditions, making them suitable for targeted high-input cultivation rather than broad adaptation. Among the hybrid crosses, a substantial number such as $L_{13} \times T_3$, $L_{14} \times T_3$, $L_{15} \times T_3$, $L_{13} \times T_1$, $L_3 \times T_2$, $L_6 \times T_2$, $L_{12} \times T_2$, $L_{13} \times T_2$ and $L_{14} \times T_2$ demonstrated above average stability, with $b_i < 1$, non-significant deviations and mean values surpassing the general mean (2.75). These crosses are promising candidates for stable dry root yield across diverse agro-climatic zones. In contrast, crosses like $L_4 \times T_1$, $L_7 \times T_1$, $L_8 \times T_1$, $L_7 \times T_2$, $L_2 \times T_3$ and $L_{12} \times T_3$ though yielding above the general mean exhibited $b_i > 1$ indicating below average stability. These may be better suited for

environments where yield potential can be maximized under optimal conditions. Among the standard checks, JA-20 stood out with a b_i value less than one and a mean exceeding the check average (4.50), confirming its above average stability and reinforcing its role as a reliable benchmark in Ashwagandha breeding trials. These findings are consistent with earlier studies by Ahmed and Dubey (2024), Pratibha *et al.* (2024) and Basser *et al.* (2025) which validate the use of regression-based stability parameters in evaluating genotype performance. The convergence of results across studies underscores the robustness of this analytical approach in identifying genotypes with desirable yield stability traits.

For the trait number of berries per plant, nearly all parental lines exhibited non-significant regression deviations, indicating a generally predictable response across diverse environments. Notably, parents T_2 , L_2 , L_3 , L_4 and L_8 showed above average stability, as evidenced by their regression coefficients less than unity ($b_i < 1$) and mean values exceeding the parental mean of 111.28. In contrast, parent T_3 displayed below average stability suggesting greater sensitivity to environmental fluctuations.

Among the hybrid crosses, a substantial number—including $L_2 \times T_3$, $L_3 \times T_3$, $L_{11} \times T_3$, $L_{13} \times T_3$, $L_{14} \times T_3$, $L_{16} \times T_3$, $L_4 \times T_1$, $L_3 \times T_2$ and $L_{12} \times T_2$ demonstrated above average stability ($b_i < 1$) and (mean > 121.75) indicating their adaptability and consistent performance across environments. These genotypes are promising candidates for cultivation under variable conditions. Conversely, crosses such as $L_6 \times T_3$, $L_9 \times T_3$, $L_{12} \times T_3$, $L_8 \times T_1$, $L_{13} \times T_1$, $L_7 \times T_2$, $L_{13} \times T_2$ and $L_{14} \times T_2$ exhibited below average stability ($b_i > 1$ and mean > 121.75) reflecting a tendency toward environmental responsiveness, which may be advantageous in targeted breeding for specific conditions but less desirable for general adaptability. Among the standard checks, both JA-134 and RVA-100 stood out with above average stability ($b_i < 1$, mean > 152.81) reinforcing their reliability and robustness under diverse agro-climatic conditions. These results underscore the value of regression-based stability analysis in identifying genotypes with consistent yield potential, and they provide a strong foundation for selecting stable performers in Ashwagandha improvement programs (Ahmed and Dubey 2024, Pratibha et al., 2024 and Bassier et al., 2025).

With respect to test weight, all parents showed non-significant deviation from regression except four, indicating their predictable response to the various environments for test weight. The parents viz., T_2 , L_2 , L_4 , L_8 and L_{11} expressed b_i values less than unity ($b_i < 1$) and a mean higher than the parental mean (2.90) indicating above average stability (Table 2b) indicating superior performance under suboptimal conditions. The parents T_1 , T_3 , L_3 , L_6 and L_7 showed b_i values greater than unity ($b_i > 1$) along with mean values above the parental mean, suggesting better performance under favorable environments but reduced stability overall. Though all crosses showed non-significant deviations from regression for test weight, the crosses $L_9 \times T_3$, $L_{14} \times T_3$, $L_{15} \times T_3$, $L_{16} \times T_3$, $L_2 \times T_1$, $L_4 \times T_1$, $L_5 \times T_1$, $L_{11} \times T_1$, $L_1 \times T_2$, $L_2 \times T_2$, $L_7 \times T_2$, $L_9 \times T_2$, $L_{11} \times T_2$ and $L_3 \times T_3$ showed non-significant deviations, b_i values less than unity ($b_i < 1$) and a mean higher than the general mean (2.85), indicating above average stability and consistent performance across environments. The crosses viz., $L_3 \times T_1$, $L_9 \times T_1$, $L_{10} \times T_1$, $L_3 \times T_2$, $L_8 \times T_2$, $L_{10} \times T_2$, $L_2 \times T_3$ and $L_7 \times T_3$ showed a mean higher than the general mean (2.85) and b_i values more than unity ($b_i > 1$), indicating below average stability suggesting sensitivity to environmental changes for test

weight. Among the checks, RVA-100 showed regression coefficient less than one ($b_i < 1$) and mean higher than the check (3.73) indicating above average stability for test weight (Bassier et al., 2025).

The evaluation of alkaloid content across parental lines and hybrid crosses revealed significant insights into genotype $\times$ environment interactions and stability dynamics. Most parental genotypes exhibited non-significant deviations from regression, indicating a generally predictable response to environmental variation. This predictability is essential for breeding programs aiming to develop stable cultivars.

Among the parental lines, genotypes T_2 , L_8 and L_{12} demonstrated above average stability, characterized by regression coefficients less than unity ($b_i < 1$) and mean values exceeding the parental average (0.36). These genotypes are likely to perform consistently under unfavourable environmental conditions making them valuable for stress-prone cultivation zones. In contrast, parents L_2 and L_3 showed below average stability, with $b_i > 1$ and higher mean values, suggesting their enhanced performance under favourable environments. Such genotypes may be best suited for high-input or controlled agricultural systems where environmental conditions are optimized. The hybrid crosses largely mirrored the parental trends, with all but nine combinations showing non-significant deviations from regression. Crosses such as $L_2 \times T_3$, $L_3 \times T_3$, $L_6 \times T_3$, $L_6 \times T_1$, $L_8 \times T_2$, $L_{14} \times T_2$ and $L_1 \times T_3$ exhibited above average stability, with $b_i < 1$ and mean values above the general mean (0.39). These combinations are promising candidates for consistent alkaloid production across diverse environments. Conversely, crosses including $L_8 \times T_1$, $L_{13} \times T_1$, $L_3 \times T_2$, $L_7 \times T_2$, $L_{12} \times T_2$, $L_5 \times T_3$, $L_{11} \times T_3$ and $L_{16} \times T_3$ showed below average stability, with $b_i > 1$ and mean values above the general mean. These genotypes may offer high alkaloid yield under favourable conditions but are less reliable in variable environments. Among the check varieties, JA-20 stood out with a regression coefficient below unity and a mean higher than the check average (0.42), confirming its above average stability and reinforcing its suitability as a benchmark genotype for alkaloid content evaluation.

The lines L_2 , L_3 , L_6 , L_{11} and L_{12} expressed non-significant deviation with b_i values less than unity ($b_i < 1$) and mean higher than the parental mean indicating above average stability and found stable under unfavorable conditions. L_3 also showed non-significant deviation with b_i values less than unity ($b_i < 1$) and mean higher than the parental mean for number of secondary and tertiary root yield. The parents T_3 and L_8 expressed b_i values more than unity ($b_i > 1$) and a mean higher than the parental mean indicating below average stability and found stable under favorable conditions for dry root yield. T_3 also showed stability under favorable conditions for number of berries per plant and test weight. The crosses such as $L_{13} \times T_3$,

$L_{14} \times T_3$, $L_{15} \times T_3$, $L_{13} \times T_1$, $L_3 \times T_2$, $L_6 \times T_2$, $L_{12} \times T_2$, $L_{13} \times T_2$ and $L_{14} \times T_2$ demonstrated non-significant deviations, b_i values less than unity ($b_i < 1$), and a mean higher than the general mean. This suggests above-average stability and suitability for unfavorable environments in terms of root yield and other yield-contributing traits. On the other hand, crosses like $L_4 \times T_1$, $L_7 \times T_1$, $L_8 \times T_1$, $L_7 \times T_2$, $L_2 \times T_3$ and $L_{12} \times T_3$ exhibited mean higher than the general mean with b_i values greater than unity ($b_i > 1$), indicating below-average stability, yet still suitable for favorable environments concerning dry root yield and related traits. The observed trends in these crosses reveal the potential of specific combinations to enhance crop performance in diverse environmental conditions. This offers valuable insights for developing effective breeding strategies. Similar results were also reported by Sangwan *et al.* (2013), Lal (2015) and Kumar *et al.* (2020).

Overall, the identification of stable genotypes—both among parents and crosses provides a strategic advantage for breeding programs. Genotypes with $b_i < 1$ and superior mean performance are particularly valuable for environments with fluctuating conditions, while those with $b_i > 1$ may be targeted for optimized cultivation systems. These findings underscore the importance of incorporating stability analysis into selection criteria to ensure consistent trait expression across diverse agro-climatic zones. These findings align with previous research and reinforce the importance of stability analysis in plant breeding programs (Eberhart and Russell, 1966 and Lal 2015).

In conclusion, stability analysis revealed that parents L_2 , L_3 , L_6 , L_{11} and 12 with non-significant deviations and b_i values less than unity ($b_i < 1$), were stable under unfavorable environmental conditions for traits such as number of secondary and tertiary roots and crosses $L_{13} \times T_3$, $L_{14} \times T_3$, $L_{15} \times T_3$, $L_{13} \times T_1$, $L_3 \times T_2$, $L_6 \times T_2$, $L_{12} \times T_2$, $L_{13} \times T_2$ and $L_{14} \times T_2$ were stable in unfavorable environments for dry root yield. Conversely, crosses like $L_{16} \times T_3$, $L_4 \times T_1$, $L_7 \times T_1$, $L_8 \times T_1$, $L_7 \times T_2$, $L_2 \times T_3$ and $L_{12} \times T_3$ were more suited to favorable environments. Therefore, these crosses may be advanced to obtain transgressive segregants and the parents of these crosses can be further utilized in hybridization programs.

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