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Delineating G × E interactions by AMMI method for root attributes in ashwagandha [*Withania somnifera* (L.) Dunal]

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Abstract

In the present study, additive main effects and multiplicative interactions (AMMI) biplot analyses was used to dissect genotype x environment interaction (GEI) and to identify location specific and widely adapted genotypes for root branches, diameter and length in ashwagandha [*Withania somnifera* (L.) Dunal]. Trials were conducted in randomized complete block design (RCBD) with two replications over three consecutive years at three different locations. ANOVA analysis revealed environment, G×E interaction and genotype effects to contribute significantly ($p<0.001$) towards total sum of squares for root branches (61.00%, 22.18% and 14.00%); root diameter (51.06%, 24.26% and 15.34%) and root length (65.67%, 20.82% and 11.39%). Further, the GEI for these traits was mostly explained by the first, second and third principal component axis (IPCA1, IPCA2 and IPCA3). AMMI1 and AMMI2 biplot analyses showed differential stability of genotypes for root branches, diameter and length with few exceptions. Environmental contribution towards the genotypic performance from AMMI1 and AMMI2 analysis for root traits except environment Bhi16 contribution for root diameter and root length. AMMI1 biplots and simultaneous selection index (SSI) statistics identified SKA-11 as the most desirable genotype for root branches and length while SKA-26 and SKA-27 for root diameter. The ashwagandha genotypes identified for root attributes could be advocated either for varietal recommendation or in varietal development program.

Key words: Ashwagandha, root traits, simultaneous selection index (SSI), principal component, GE interaction

Introduction

Since the start of human civilization herbal plants are considered as one of the most important sources of medicines. Approximately 25% of all contemporary medicines are derived, directly or indirectly from medicinal plants, primarily through the application of modern technology and traditional knowledge. In certain cases of pharmaceuticals, such as antitumoral and antimicrobial medicines, this percentage may be as high as 60% (Calixto 2000; Sucher and Carles 2008). World Health Organization (WHO), reported the international market of herbal products is around $6.2 billion, which is perched to grow to $5 trillion by the year 2050. Ashwagandha [*Withania somnifera* (L.) Dunal] also known as Indian Ginseng or winter cherry belongs to the family Solanaceae. It is native to India and cultivated throughout the drier and subtropical parts of India. Ashwagandha is one of the most valued medicinal plants, used in more than 100 formulations of Ayurveda and is thought to be therapeutically equivalent to Ginseng (Sangwan et al. 2004). The plant is also known to trigger the immune system cells namely, lymphocytes and phagocytes, which also help to control the effects of stress and promote general wellness and other ailments (Singh et al. 2001).

The roots are considered as the most important part of the plant as main categories of constituents are considered to be of therapeutic importance. Phytochemicals from root extracts have antiviral activity and may be effective in controlling the viral infections (Balkrishna et al. 2020). The root quality of ashwagandha is determined by its alkaloid contents and textural attributes (Ramesh Kumar et al. 2012), which helps in identification of desirable genotypes and determining the market value.

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Stability is one of the important criteria in any breeding programme and this issue can be addressed by phenotypic expression of traits in targeted environment (Rad et al. 2013). A morphological variation in genotypes is highly dependent on the environments and interaction of genotypes with environments. Explaining such variations is biased upwardly by the fact that all genotypes don't react in the same way as change in circles and the two environments do not have exactly the same conditions (Neisse et al. 2018). Therefore, combined analysis of any variance is required that can measure genotype environment interaction (GEI) and identify prime component, though it is not sufficient to declare the GEI effectiveness. Several statistical methods have been proposed for analysis of plant stability with the aim of dissecting GEI and stable trait expression across environments. These models are used to assess the adaptability and stability of genotypes across the environments (Yan and Tinker 2006). Limited work is done by Sangwan et al. (2013) and Kumar et al. (2020) for various traits in ashwagandha using Eberhart & Russell (1966) model.

AMMI is potential tool to evaluate MET data in order to interpret complex GEI interaction (Yan and Tinker 2006). It can effectively depict the interaction pattern graphically and delineate the environments to evaluate the various genotypes (Yan et al. 2007). However, limited studies have been carried out to analyze the MET using this potential tool. Further, AMMI model does not make provision for quantitative stability measure so one stability measure known as AMMI stability index (ASI) and simultaneous selection index (SSI) for yield and stability are used by researchers to simultaneously select high yielding and stable genotypes. Considering the effectiveness and reliability of AMMI model alongwith SSI to select genotypes with higher yield and better stability in terms of root branches, diameter and length, ashwagandha genotypes were evaluated under varying environmental conditions. Therefore, a study was conducted to dissect GEI for yield and component traits in 16 ashwagandha genotypes using AMMI analysis and to identify high yielding and stable genotypes across the environments (years) for future use in crop improvement programme.

Materials and methods

Sixteen genotypes of ashwagandha were taken for study from S. D. Agricultural University, S. K. Nagar, Gujarat, India including one check (AWS-1). The information on location, year, environmental code, genotype and their pedigree is given in Table 1. Trials were conducted over three consecutive years, 2016-17, 2017-18 and 2018-19 at three different locations (S.K.Nagar, Jagudan and Bhiloda) in the semi-arid conditions of Gujarat. The experiment was laid down in a randomized complete block design (RCBD) with two replications. Seeds were sown directly in third week of October in each year and thinned 45 days after sowing. Each genotype was sown in 2 m long rows with total number of 4 rows per plot per replication. The row-to row and plant-to-plant distance of 30 cm and 10 cm respectively, were maintained in each plot. The roots were dug 150 days after sowing, washed with tap water, and dried in the hot air oven for 48 hours. In each plot, five competitive plants from each replication were randomly selected to record the observations on root branches, diameter and length. In total, nine experimental trials were considered as environments to analyze GEI.

Table 1. List of genotypes, their pedigree, year of experiments and locations

S.No.	Genotype code	Pedigree	S.No.	Genotype code	Pedigree	Environment code	Year	Location
1	SKA-1	MWS-316-2-1-3	9	SKA-19	RAS-33-1-1-4	Skn16	2016-17	S.K.Nagar
2	SKA-3	MWS-226-2-2-1	10	SKA-21	RAS-55-4-2-3	Skn17	2017-18	S.K.Nagar
3	SKA-4	MWS-205-3-2-1	11	SKA-23	RAS-29-3-1-2	Skn18	2018-19	S.K.Nagar
4	SKA-6	MWS-322-2-2-3	12	SKA-24	MPAS-3-3-1-4	Jag16	2016-17	Jagudan
5	SKA-10	MWS-309-3-1-1	13	SKA-25	MPAS-4-1-2-3	Jag17	2017-18	Jagudan
6	SKA-11	MWS-101-3-2-1	14	SKA-26	MPAS-5-4-1-3	Jag18	2018-19	Jagudan
7	SKA-12	MWS-208-3-1-2	15	SKA-27	MPAS-7-1-1-4	Bhi16	2016-17	Bhiloda
8	SKA-17	RAS-23-2-1-4	16	AWS-1	Check	Bhi17	2017-18	Bhiloda
						Bhi18	2018-19	Bhiloda

The AMMI model was used to analyse the GEI. The AMMI model for the i^{th} genotype in the j^{th} environment was followed as per Zobel et al. (1988):

$$Y_{ijr} = \mu + g_i + e_j + b_r(e_j) + \sum_{k=1}^{n} \lambda_k \alpha_{ik} \gamma_{jk} + \rho_{ij} + \varepsilon_{ij}$$

where, Y_{ijr} is the yield of root parameters of genotype *i* in environment *j* for replicate *r*, μ is the grand mean, g_i is the deviation of genotype *i* from the grand mean, e_j is the environment main effect as deviation from μ, λ_k is the singular value for the interaction principal component (IPC) axis *k*, α_{ik} and γ_{jk} are the genotype and environment IPC scores (*i.e.* the left and right singular vectors) for axis *k*, $b_r(e_j)$ is the effect of the block *r* within the environment *j*, *r* is the number of blocks, ρ_{ij} is the residual containing all multiplicative terms not included in the model, *n* is the number of axes or IPC that were retained in the model, and ε_{ij} is error under independent and identically distribution assumptions.

The AMMI stability index (ASI) as described by Jambhulkar (2014) was calculated as follows:

$$ASI = \sqrt{\left[PC_1^2 \times \theta_1^2\right] + \left[PC_2^2 \times \theta_2^2\right]}$$

where, PC_1 and PC_2 are the scores of 1^{st} and 2^{nd} IPCs respectively; and θ_1 and θ_2 are percentage sum of squares explained by 1^{st} and 2^{nd} principal component interaction effect respectively. The larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller ASI scores indicate a more stable genotype across environments.

Simultaneous stability index (SSI) incorporate mean and stability index in a single criteria and calculated as: SSI = rASI + rY where, rASI is the rank of ASI and rY is the rank of mean yield of genotypes across environments. This index considered the rank of AMMI stability index (ASI) and rank of genotypes based on root traits across environments (Farshadfar et al. 2011). The AMMI and stability indices were determined using R statistical software, version 3.4.1 (R Development Core Team 2018).

Results and discussion

AMMI analysis of variance

The AMMI model retrieves the part of the sum of squares that determines the G × E interaction which is called the standard portion (the genotype and environment effect) and a residual part which corresponds to unpredictable and uninterruptable responses from the model (Cornelius et al. 1996). In the present study, the responses of ashwagandha genotypes to environmental conditions were investigated by the AMMI model based on variations in root attributes. The ANOVA of AMMI biplot analysis indicated variability due to the environmental and interaction effects was greater than the variability caused by genotype effects (Table 2). Genotype, environment and GEI effects were highly significant ($p<0.001$) for root branches, revealing the presence of variability among genotypes as well as environments under which experiments were undertaken. Main effect of environments represented about 61.00% of the total variation, whereas G × E interactions and genotype effects represented 22.18% and 14.00% respectively. For root diameter significance of 51.06%, 24.26% and 15.34% sum of squares ($p<0.001$) belonged to environment, interaction effects and genotype respectively. Further, the results also indicated that 65.67% of the variation was due to environment effects, whereas 20.82% belonged to GE effects and 11.39% to genotypic effects for root length. Hence, environment contributed more to the total variation in root characteristics than the G and GE. It indicated the significant differences between the averages of environments and their interaction with genotypes, which caused most of the variations accounted. Similar results were observed in groundnut (Oliveira and Godoy 2006; Ajay et al. 2019), yellow passion fruit (Oliveira et al. 2009), cassava (Adjebeng-Danquah et al. 2017), chickpea (Alemu et al. 2017), triticale (Kendal et al. 2019) and wheat (Neisse et al. 2018). Most of the existing variations were explained by environment and G x E interaction effects, that makes the selection difficult for the breeder. The significance of environmental effect indicated that the environments under study were variable and selecting the suitable genotypes in multilocation experiment is tedious process. Contrary to these results, AMMI analysis of seed yield and mucilage content in *Plantago* (Shahriari et al. 2018) and groundnut (Kumar et al. 2019) reported genotypic effects to be predominant source of variation.

Further, the sum of squares due to G x E interaction for root branches, diameter and length traits was mainly explained by the first, second and third interaction principal component axis (IPCA1, IPCA2 and IPCA3) with 84.13%, 12.76% and 3.11%; 37.60%,

25.40% and 17.40%; 61.80%, 21.00% and 12.70% of GEI sum of squares respectively (Table 2). So, in the present study AMMI having three interaction principle components was the best predictive model. Oliveira and Godoy (2006); Ajay et al. (2019) in groundnut, Neisse et al. 2018 in wheat and Kendal et al. 2019 in triticale have also reported environment and interactions as a predominant source of variation for studied traits.

Mean performance and stability of genotypes

Development of new genotypes with high yield and acceptable level of stability is one important breeding programme. The genotypic mean, ASI, SSI and relative rankings of genotypes on the basis of yield and stability are presented in table 3. AMMI stability indices (ASI) rank the genotypes on the basis of yield stability. Less is the value of ASI the more stable is the genotype and low is GEI (Purchase 2000). Results of ASI showed that genotype, SKA-21 was most stable for root branches and root length while, SKA-23 was most stable for root diameter across environments. However, ASI only gives an idea about the stability of genotypes but it does not provide any information about mean performance. So, simultaneous selection index (SSI), was computed as per formula suggested by Farshadfar et al. (2011) by adding the ranks of stability parameter and average yield. The least SSI is considered as most stable with high yield, whereas high SSI is considered as least stable with low yield (Mohammadi and Amri 2008; Farshadfar et al. 2011). The mean rank of each genotype based on SSI scores is presented in Table 3. Based on SSI, SKA-11, SKA-21, SKA-10, SKA-26 and SKA-23 were the desirable genotypes for root branches, SKA-26, SKA-27, SKA-23, SKA-12 and SKA-17 for root diameter and SKA11, SKA-3, SKA-17, SKA-6 and SKA-19 for root length. The similar statistics were used by Adjebeng-Danquah et al. (2017) in cassava; Alemu et al. (2017) in chick pea; Shahriari et al. (2018) in *Plantago*; Ajay et al. (2019) and Kumar et al. (2019) in groundnut to delineate the desirable genotypes for various traits. Conclusively, genotype SKA-11 was identified as most stable and high yielder for root branches and root length (Figs. 2a, 2b and 2c) while, SKA-26 and SKA-27 were most desirable for root diameter.

Further stability of genotypes is evaluated in the Y-axis (IPCA1) by AMMI1, while AMMI2 analysis reported stable environments and genotypes located near the origin, with low scores for the two axes of the interaction (IPCA1 and IPCA2). Accordingly, AMMI1 analysis revealed, genotypes SKA-11, SKA-26 and SKA-27 were the most stable, as indicated by values near the origin of the IPCA1 axis, which is indicative of a smaller contribution to the G × E interaction. From similar analysis, genotypes SKA-21, SKA-10, SKA-12 and SKA-1 were most stable for root branches (Fig. 1a) and for root diameter genotypes SKA-27 and SKA-26 were high yielder and most stable (Fig. 1b). The genotypes SKA-11, SKA-17 and SKA-6 were highly desirable for root length (Fig. 1c). Similar grouping of genotypes as desirable, stable and unstable have been reported in *Plantago* by Shahriari et al. (2018), in wheat by Neisse et al. (2018) and in triticale by Kendal et al. (2019).

Based on the AMMI2 analysis, genotypes SKA-27, SKA-26 and SKA-11 were the most desirable genotypes for root branches as they possessed high stability and better yield. SKA-21, SKA-10, SKA-12 and SKA-1 were found to be most stable for root branches. Moreover, genotype, SKA-25 showed adaptability specific to environments, Skn16 and Jag16 and SKA-24 to Bhi16 for root branches (Fig. 1d). For root diameter SKA-26 was the most desirable genotype although SKA-17 and SKA-23 had high stability. SKA-10 showed specific adaptability to environments Jag16 while, SKA-4 to Jag17 for root diameter (Fig. 1e). Genotype SKA-12 was high yielder and most stable; SKA-21 was most stable while, AWS-1, SKA-26 and SKA-19 were highly unstable for root length. SKA-24 and SKA-27 showed specific adaptability to environment Bhi16 and SKA-10 were specifically adapted to environment Jag16 (Fig. 1f). Similarly, genotypes for wide and specific adaptation for different traits were identified by Neisse et al. (2018) in wheat, Shahriari et al. (2018) in *Plantago* and Kendal et al. (2019) in triticale.

Environmental stability is important for demonstrating the reliability of genotype ordering in a given environment in relation to the rating for the environments undertaken. The AMMI1 biplot graph showed environments Skn16, Jag16 and Bhi16 as the main contributors to the phenotypic stability of the genotypes for root branches (Fig. 1a). For root diameter, environments Jag17, Bhi17 and Jag16 contributed less to stability and more to GE interaction and environments Skn16, Bhi16, Skn17, Skn18, Jag18 and Bhi18 were the largest contributor towards the stability of genotypes (Fig. 1b). All the environments contributed largely towards the stability of genotypes for root length except Jag16 (Fig. 1c). Environmental

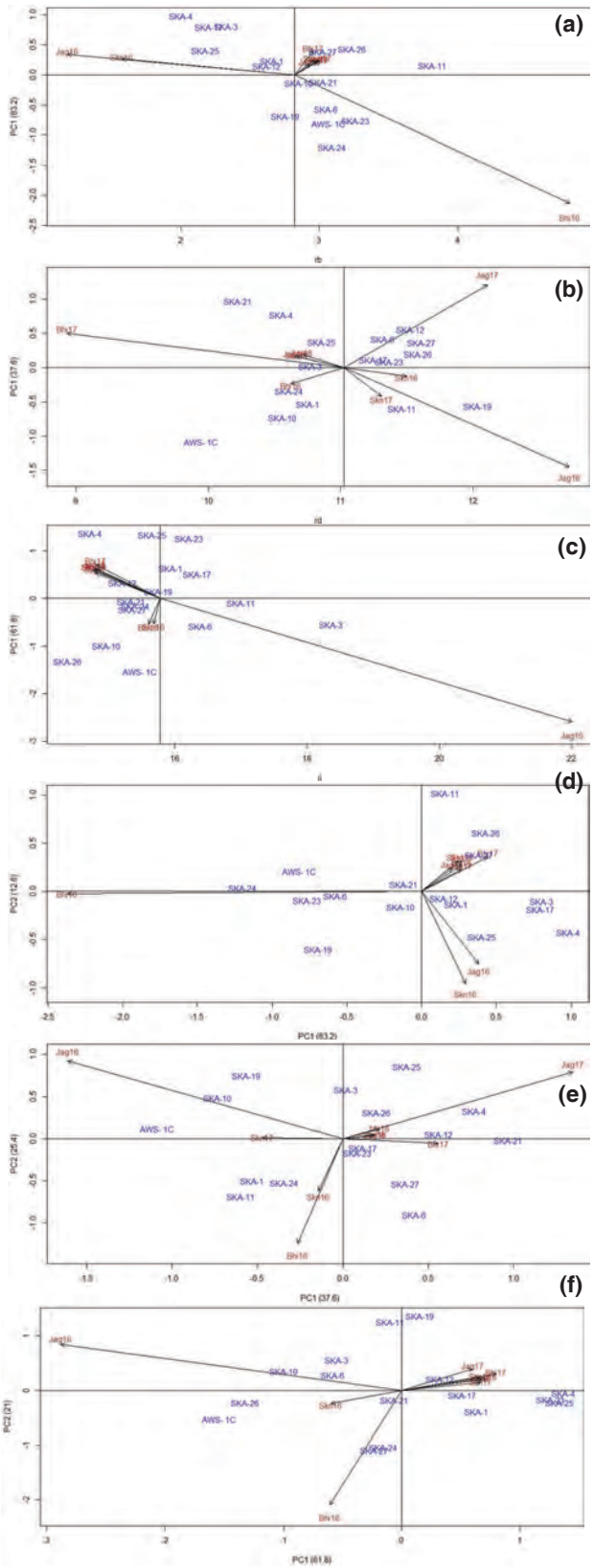

Fig. 1. AMMI biplot showing AMMI1 for a. root branches, b. root diameter c. root length and AMMI2 for d. root branches, e. root diameter and f. root length of 16 ashwagandha genotypes

A: SKA-11 Vegetative stage

B: SKA-11 Reproductive stage

C: SKA-11 Maturity stage (root part)

Fig. 2. Diagram showing desirable ashwagandha genotype SKA-11 at a. Vegetative stage, b. Reproductive stage and c. Maturity stage

Table 2. AMMI analysis of variance of root traits for 16 ashwagandha genotypes

Sources of variation	DOF	SS	MSS	F value	Pr (>F)	% explained
			Root branches			
Environment (E)	8	268.32	33.54	1717.52	2.10E-13***	61.00
Rep (E)	9	0.18	0.02	0.22	0.9918	0.04
Genotype (G)	15	61.59	4.11	45.36	< 2.2e-16***	14.00
G × E interaction	120	97.56	0.82	8.99	< 2.2e-16***	22.18
IPCA1	22	81.15	3.69	40.75	0.0***	84.13
IPCA2	20	12.31	0.62	6.80	0.0***	12.76
IPCA3	18	2.99	0.17	1.84	0.0264*	3.11
Residuals	135	12.22	0.091			2.78
			Root diameter (mm)			
Environment (E)	8	297.59	37.20	39.86	3.93E-06***	51.06
Rep (E)	9	8.40	0.94	2.74	0.00578**	1.44
Genotype (G)	15	89.40	5.96	17.47	< 2.2e-16***	15.34
G × E interaction	120	141.39	1.18	3.46	3.77E-12***	24.26
IPCA1	22	53.10	2.41	7.07	0.0	37.60
IPCA2	20	35.93	1.80	5.27	0.0	25.40
IPCA3	18	24.58	1.37	4.00	0.0	17.40
Residuals	135	46.055	0.341			7.90
			Root length (cm)			
Environment (E)	8	1428.63	178.58	1226.88	9.51E-13***	65.67
Rep (E)	9	1.31	0.15	0.44	0.9135	0.06
Genotype (G)	15	247.69	16.51	49.48	< 2.2e-16***	11.39
G × E interaction	120	452.87	3.77	11.31	< 2.2e-16***	20.82
IPCA1	22	279.76	12.72	38.10	0.0	61.80
IPCA2	20	95.07	4.75	14.24	0.0	21.00
IPCA3	18	57.53	3.20	9.58	0.0	12.70
Residuals	135	45.05	0.33			2.07

DOF = Degree of freedom; SS = Sum of squares; MSS = Mean sum of squares; IPCA = Interaction Principal Component Analysis Axis; Significance codes: '***' = 0.001, '**' = 0.01, '*' = 0.05

contribution towards the genotypes stability from AMMI2 biplot graphs was in correspondence with AMMI1 for root branches (Fig. 1d). For root diameter Bhi16, Jag16 and Jag17 contributed less while other environments more towards the stability (Fig. 1e). For root length, Bhi16 and Jag16 contributed comparatively high to the GE interaction whereas remaining environments contributed more towards the stability of genotypes (Fig. 1f). The contribution of AMMI2 to GEI sum of squares was in conformity with the previous study of Neisse et al. 2018 in wheat and Shahriari et al. 2018 in *Plantago* and Kendal et al. 2019 in triticale. They showed that AMMI2 biplot may be more accurate to extract GEI variation as it contains information of two IPCAs and greater pattern proportion compared to the AMMI1. AMMI2 model is simple and elucidates the stability, genotypic performance, genetic variance between genotypes, and the environments that optimize varietal performance (Miranda et al. 2009). In the AMMI2 biplot graph, similar genotypes and environments have positive associations and placed near the origin of biplot of stable genotypes (Silveira et al. 2013). In the present investigation, AMMI2 biplot showed genotype SKA-21 to be highly stable for both root branches and root length while SKA-26 as most desirable *i.e.* higher

Table 3. Average number of root branches, root diameter and root length of ashwagandha (Y) and other stability parameters: Additive Main effects and Multiplicative Interaction (AMMI) stability Index (ASI), rankings of mean value (rY) and ASI (rASI) and Simultaneous Selection Index (SSI)

S.No.	Genotype	Root branches					Root diameter (mm)					Root length (cm)				
		Y	rY	ASI	rASI	SSI	Y	rY	ASI	rASI	SSI	Y	rY	ASI	rASI	SSI
1	SKA-1	2.66	11	0.19	5	16	10.75	11	0.252	7	18	15.95	6	0.40	9	15
2	SKA-3	2.33	13	0.67	13	26	10.77	10	0.166	4	14	18.37	1	0.38	8	9
3	SKA-4	2.00	16	0.82	15	31	10.55	14	0.303	12	26	14.72	15	0.85	15	30
4	SKA-6	3.05	6	0.48	9	15	11.32	7	0.293	10	17	16.39	3	0.42	10	13
5	SKA-10	2.85	9	0.12	2	11	10.56	13	0.314	13	26	14.96	14	0.62	11	25
6	SKA-11	3.82	1	0.18	4	5	11.46	5	0.302	11	16	17.00	2	0.27	5	7
7	SKA-12	2.62	12	0.12	3	15	11.53	4	0.218	6	10	15.21	13	0.22	2	15
8	SKA-17	2.20	14	0.66	12	26	11.25	8	0.106	2	10	16.34	4	0.31	7	11
9	SKA-19	2.75	10	0.58	10	20	12.04	1	0.328	14	15	15.75	7	0.30	6	13
10	SKA-21	3.03	7	0.10	1	8	10.22	15	0.367	15	30	15.34	12	0.06	1	13
11	SKA-23	3.26	2	0.64	11	13	11.37	6	0.072	1	7	16.22	5	0.78	12	17
12	SKA-24	3.09	4	1.00	16	20	10.61	12	0.276	9	21	15.40	10	0.24	3	13
13	SKA-25	2.17	15	0.34	7	22	10.86	9	0.273	8	17	15.66	8	0.824	14	22
14	SKA-26	3.23	3	0.36	8	11	11.58	3	0.113	3	6	14.38	16	0.821	13	29
15	SKA-27	3.02	8	0.32	6	14	11.61	2	0.209	5	7	15.36	11	0.27	4	15
16	AWS-1	3.07	5	0.68	14	19	9.94	16	0.411	16	32	15.48	9	0.96	16	25

yields and stability for root branches and root diameter. Genotype SKA-24 showed specific adaptation to environment Bhi16 for root branches and root length. Similarly, SKA-10 was specifically adapted to the environment Jag16 for root diameter and length. SKA-27 was most desirable for root branches but showed specific adaptation for root length to environment Bhi16. This showed differential response of tested genotypes for different root attributes and adaptation in ashwagandha.

The AMMI model is effective as it contribute to a larger portion of the GEI sum of squares and separate the main and interaction effects. In the current study, SSI statistics and AMMI1 biplot model had similar results for root attributes with few exceptions (SKA-27 for root branches and SKA-19 for root length) in sight of genotype stability. However, similar and differential response was obtained for general and specific adaptation of genotypes to environment from AMMI1 and AMMI2 models. Moreover, similar results were obtained for environmental contribution towards the genotypes from AMMI1 and AMMI2 analysis for root traits except environment, Bhi16 for root diameter and root length. This indicated that the traits are governed by different sets of genes and effect of environment on the cumulative expression of different set of genes will vary considerably, which is observed as variation in stability of genotypes for root branches, diameter and length.

Root attributes were highly affected by environment and G x E interactions. Because of the greater phenotypic stability the Skn16 environment can be used during the initial stages of selecting new genotypes. Conclusively, on the basis of AMMI1 and AMMI2 analysis, ASI and SSI scores, SKA-11 was identified as most stable for root branches and length while SKA-26 and SKA-27 for root diameter, when considering the average over three consecutive years at three different locations in addition to their high yield potential. AMMI stability analysis showed that environments were dissected into favourable and unfavourable for average root branches, diameter and root length. Location-specific adaptation of genotypes as reported in the present study clearly suggested that location-specific breeding needs to be undertaken along with focusing on wider adaptability. In this regard, futuristic participatory plant breeding as well as present research station oriented breeding program should be

advocated for the ashwagandha improvement. In addition, in the existing procedure of varietal release, average of a given genotype over years and/or locations, and its superiority over the checks is only considered, while stability of genotypes is overlooked. Presence of significant cross over interactions obviously suggests that the existing system does not logically represent the actual situation. Rather, efforts are necessary to identify location-specific genotypes over the years and locations to consider them for their release, since this will take into consideration the stability parameter of the genotypes.

Author's contribution

Conceptualization of research (MK, MPP); Designing of the experiments (MK, MPP, SDS); Contribution of experimental materials (MK, RMC, CJT, NVS, PCP, PTP, HSB, NBP); Execution of field/lab experiments and data collection (MK, RAG, RMP); Analysis of data and interpretation (MK, MPP, KR); Preparation of manuscript (MK, KR).

Declaration

The authors declare no conflict of interest.

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