



# Evaluation of Indian ginseng [*Withania somnifera* (L.) Dunal] breeding lines and genotype-by-environment interaction across production environments in western India

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## Abstract

In order to find location-specific and broadly adapted genotypes for total root alkaloid content and dry root yield along with additive main effects and multiplicative interactions (AMMI) and genotype (G) main effects plus genotype × environment (E) interaction in Indian ginseng (*Withania somnifera* (L.) Dunal), (GGE) biplot analyses were used in the current study. Trials were carried out in a randomized complete block design (RCBD) over three succeeding years viz., 2016–2017, 2017–2018 and 2018–2019 at three different locations (S. K. Nagar, Bhiloda and Jagudan). Analysis of variance (ANOVA) for AMMI for dry root yield revealed that the environment, genotype, and GE interaction, respectively, accounted for significant sums of squares of 35.31%, 24.89%, and 32.96%. For total root alkaloid content, a significance of 27.59% of total sum of squares was justified by environment, 17.72% by genotype and 43.13% by GEI. Nine experimental trials in total were taken into consideration as contexts for the GEI analysis in 16 genotypes, including one check. AMMI analysis showed that genotypes, SKA-11, SKA-27, SKA-23 and SKA-10 were superior for mean dry root yield and SKA-11, SKA-27 and SKA-21 had better performance for total root alkaloid content across environment. The GGE biplot analysis showed genotypes SKA-11, SKA-27, SKA-10 desirable for dry root yield and SKA-26, SKA-27, SKA-11 for total root alkaloid content. As a result of the GGE and AMMI biplot techniques, SKA-11 and SKA-27 were determined to be the most desired genotypes for both total root alkaloid content and dry root yield. Further, simultaneous stability index or SSI statistics identified SKA-6, SKA-10, SKA-27, SKA-11 and AWS-1 for higher dry root yield, whilst SKA-25, SKA-6, SKA-11, SKA-12 and AWS-1 for total alkaloid content from root. Based on trait variation, GGE biplot analysis identified two mega-environments for dry root yield and a total of four for total root alkaloid content. Additionally, two representative and discriminating environments—one for dry root production and the other for total root alkaloid content were found. Location-specific and breeding for broad adaptation could be advocated for improvement and release of varieties for Indian ginseng.

**Keywords** Indian ginseng · Dry root yield · Total root alkaloid content · Mega-environments · Simultaneous stability index · Discriminating and representative environments

## Introduction

One of the most valuable medicinal plant species is *Withania somnifera* (L.) Dunal, also known as “Indian ginseng”, “Indian Winter Cherry”, or “Ashwagandha”. The plant has been classified as a rejuvenator and is frequently used to increase vigour, physical endurance, strength, and vitality. It has been used for a very long time in the traditional Indian system of medicine. Phytochemicals from roots have antiviral properties and may be useful in treating COVID-19 infection (Srivastava et al. 2020; Kumar et al. 2021).

While the usage of herbal medicines is expanding, the breeding of medicinal plants is receiving less attention.

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Climate change is currently the biggest danger to quantity and quality of all agricultural products. Environmental factors impacting plant development and productivity should be better understood and regulated in order to increase and stabilise genetic advancements (Xu 2016). Finding environmental factors and high-yielding stable genotypes across habitats is a fundamental strategy for developing new kinds. Genotype multilocation testing may be helpful in this situation to examine how genotypes interact with target environment and function consistently across a variety of environment. The commercial output of around 75% of medicinal plants, which are wild species or wild relatives, is significantly impacted by environmental conditions (Carter et al. 2005). One can predict the synthesis of secondary metabolites and their stability across environment just by selecting stable genotypes; process that produces a population acclimated to the right growing conditions (Yan and Tinker 2006).

Sum of squares that AMMI model retrieves is the effect of genotype and environment, and a residual fraction that accounts for unpredictable and unavoidable reactions is the part that determines the GE interaction (Cornelius et al. 1996). By extending AMMI Biplot's environmental classification, GGE Biplot makes it possible to identify mega-environments and the best performing genotypes in these groups (Miranda et al. 2009). GGE biplot utilize PCA approach to examine the data from multi-environment and enable visual representation of association among G, E, and GE interactions (Kendal et al. 2019). AMMI model extracts the genotype and environment effect from sum of squares, and a residual part, which corresponds to unpredictable and uninterruptable reactions, is the part that finds out GE interaction (Cornelius et al. 1996).

## Materials and methods

### Experimental locations

Trials were conducted over the course of three consecutive years, 2016–2017, 2017–2018 and 2018–2019 at three different locations viz., S. K. Nagar, Bhiloda and Jagudan in the semi-arid conditions of Gujarat, India. Each planting year was considered as an environment, thus a total of nine environment designated as Skn16, Bhi16, Jag16, Skn17, Bhi17, Jag17, Skn18, Bhi18, Jag18 were formed.

### Experimental materials and design

The experimental material comprised of 16 diverse genotypes of Indian ginseng including one check (AWS-1). The genotypes viz., SKA-1, SKA-3, SKA-4, SKA-6, SKA-10, SKA-11, SKA-12, SKA-17, SKA-19, SKA-21, SKA-23,

SKA-24, SKA-25, SKA-26 and SKA-27 represented diverse genetic stocks of *Withania* from different regions of India. The experiment was conducted in a randomized complete block design (RCBD) with two replications for each environment.

### Estimation of dry root yield (g) and total root alkaloid content (%)

The roots were dug about 150 days after sowing, rinsed in running water, and dried for 48 h in the hot air oven. The roots were placed in an airtight polythene bag and dried till their moisture content was 7–8% of its initial level before being used for biochemical analysis. Five competitive plants from each replication from each plot were chosen at random, and their dry root yield per plant (g) and total root alkaloid content (%) observations were recorded. According to the methodology described by Kumar et al. (2020a), the alkaloid content in percentages from adequately dried root samples from all genotypes were calculated.

## Statistical analysis

### Analysis of AMMI model

AMMI model suggested by Zobel et al. (1988) for the  $i$ th genotype in the  $j$ th environment is.

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

where,  $Y_{ijr}$  is the total root alkaloid content or dry root yield for genotype  $i$  in environment  $j$  for replicate  $r$ ,  $\mu$  is the grand mean,  $g_i$  is the deviation of genotype  $i$  from grand mean,  $e_j$  is the main effect of environment as deviation from grand mean,  $\lambda_k$  is the singular value for the interaction principal component (IPC) axis  $k$ ,  $\alpha_{ik}$  and  $\gamma_{jk}$  are IPC scores for axis  $k$ , for genotype and environment (i.e. the left and right singular vectors),  $b_r(e_j)$  is the block effect  $r$  in the environment  $j$ ,  $r$  is the total number of blocks,  $\rho_{ij}$  is the residual containing all multiplicative terms not included in the model,  $n$  is total number of axes or IPC that were retained in the model, and  $\varepsilon_{ij}$  is the error under assumptions of independent and identical distribution.

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

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

where,  $PC_1$  and  $PC_2$  are first and second IPCs scores respectively; and  $\theta_1$  and  $\theta_2$  are % sum of squares explained by first and second principal component interaction effect, respectively. Larger the IPCA score, either negative or positive, the

more specifically adapted a genotype is to particular environments. A genotype that is more stable across contexts has lower ASI score.

Simultaneous stability index or SSI include mean yield and stability index in a single criterion and computed as:  $SSI = rASI + rY$  where,  $rASI$  is the rank of ASI and  $rY$  is the rank assigned according to the mean yield of genotypes across environments. This index considered the rank of AMMI stability index (ASI) and rank of genotypes based on yield across environments (Farshadfar et al. 2011).

### GGE biplot analysis

Singular value decomposition (SVD) of the first two principal components was used to fit the GGE biplot model (Yan 2002),

$$Y_{ij} = \mu + \beta_j + \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where,  $Y_{ij}$  is the trait mean for genotype  $i$  in environment  $j$ ,  $\mu$  is the grand mean,  $\beta_j$  is the main effect of environment  $j$ ,  $\mu + \beta_j$  being the mean yield across all genotypes in environment  $j$ ,  $\lambda_1$  and  $\lambda_2$  are the singular values (SV) for the first and second principal components ( $PC_1$  and  $PC_2$ ), respectively,  $\xi_{i1}$  and  $\xi_{i2}$  are eigen vectors of genotype  $i$  for  $PC_1$  and  $PC_2$ , respectively,  $\eta_{j1}$  and  $\eta_{j2}$  are eigen vectors of environment  $j$  for  $PC_1$  and  $PC_2$ , respectively,  $\varepsilon_{ij}$  is the residual associated with genotype  $i$  in environment  $j$ . In GGE biplot analysis, scores of  $PC_1$  were plotted against  $PC_2$  (Yan and Tinker 2006). R statistical software, version 3.4.1, was used to conduct the AMMI and GGE biplot studies (R Development Core Team, 2021).

## Results

### AMMI analysis of variance

Results of the current AMMI study showed that genotypic impact scores were somewhat more dispersed than the environmental effect scores, showing that genotypic variability outweighs environmental variability (Fig. 1A, B). AMMI analysis for dry root yield over the environment in the semi arid conditions of Gujarat depicted that 35.31%, 32.96% and 24.89% significant sum of squares was explained by the environment,  $G \times E$  interaction and genotype, respectively (Table 1). Further, for total root alkaloid content, significance of 43.13% was justified by GEI, 27.59% by environment and 17.72% by genotype. Furthermore, first principle component axis (IPCA1) contributed 80.4% of the GEI sum of squares for dry root yield and first three IPCAs together provided 97.8% of the GEI variation and were highly significant (Table 1). While IPCA1, IPCA2, and IPCA3 accounted

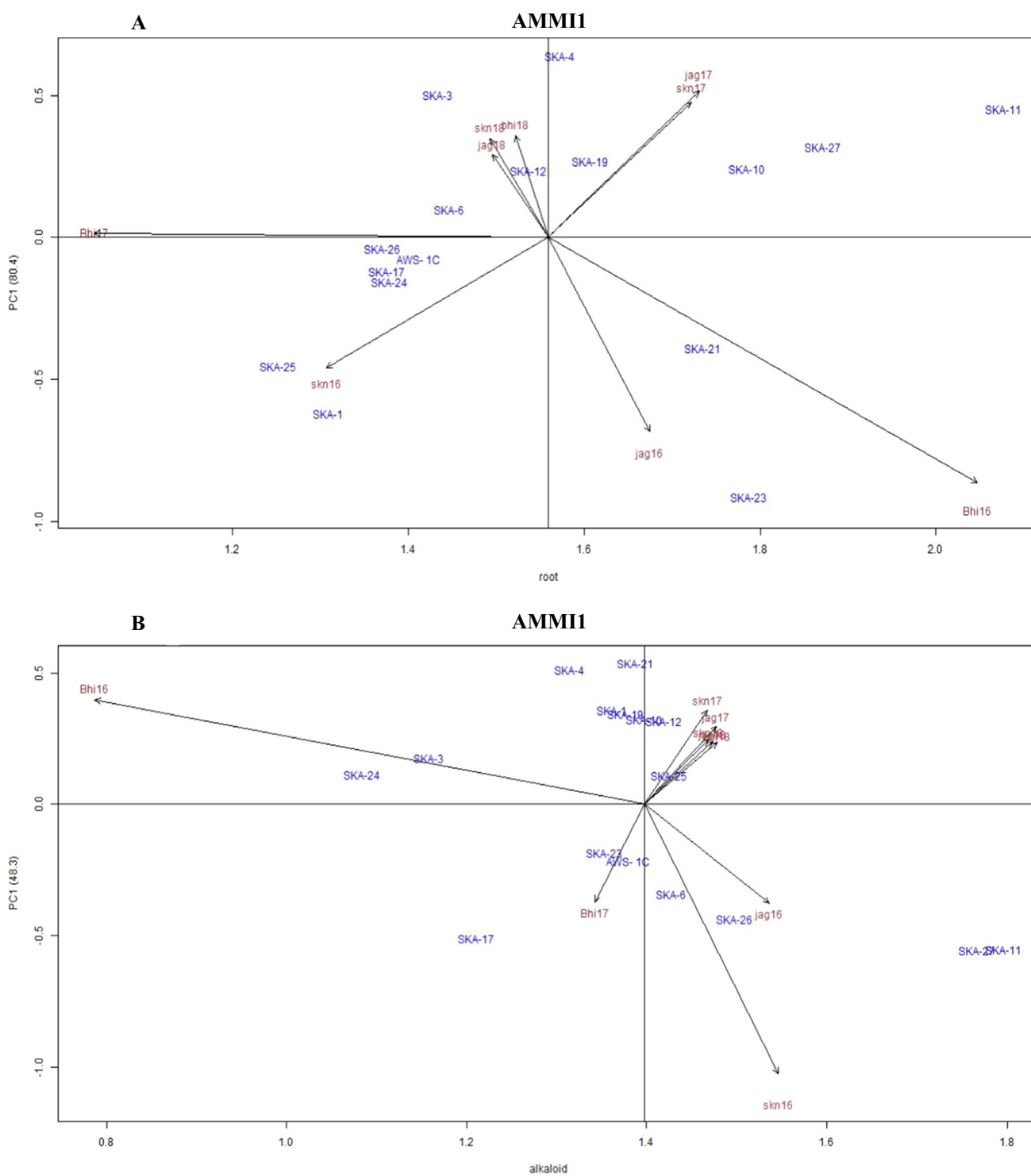
for 48.3%, 28.1%, and 19.6% of the variation for total root alkaloid content, respectively, other principal components were not statistically significant (Table 1).

### Stability, adaptability and mean genotypic performance

The genotypic mean, SSI, ASI, IPCA1, IPCA2 scores and relative rankings of genotypes on the basis of yield and stability are presented in Table 2. More stable the genotype, lower the ASI value, and consequently, lower the GEI value (Purchase et al. 2000). So, SKA-26, SKA-6, AWS-1, SKA-17 and SKA-24 were reported as most stable genotypes for dry root yield based on ASI values while, SKA-25, SKA-3, AWS-1, SKA-24 and SKA-12 were most stable genotypes for total root alkaloid content. SSI is a index that consider the rank of ASI to rank genotypes (Mohammadi and Amri 2008; Farshadfar et al. 2011). SSI depicts genotypic superiority as smaller SSI value indicates higher general or wide adaptation. Based on SSI, SKA-10, SKA-6, SKA-27, SKA-11 and AWS-1 were chosen as the best for dry root production, whilst SKA-25, SKA-6, SKA-12, AWS-1 and SKA-11 for total root alkaloid content (Table 2).

Environmental means, IPCA1 and IPCA2 scores of environment and comparative rankings of top three genotypes based on mean performance for dry root yield and total alkaloid content in root are listed in Table 3. From AMMI analysis, SKA-11, SKA-27, SKA-23 and SKA-10 were reported superior for mean dry root yield along with SKA-11, SKA-27 and SKA-21 genotypes for total root alkaloid content in the environment studied. The mean comparison of environment showed that Bhi16, Jag17, Skn17 and Jag16 environment had the highest average dry root yield while; lowest average yield was obtained from Bhi17 and Skn16 environment. Overall, the year 2016–2017 of experiment imparted highest dry root yield (1.68 g) followed by 2017–2018 (1.507 g) and 2018–2019 (1.506 g). While 2016–2017 had the lowest mean of 1.29%, the experimental years 2017–18 and 2018–2019 contributed equivalent and higher average total root alkaloid contents of 1.43% and 1.47%, respectively.

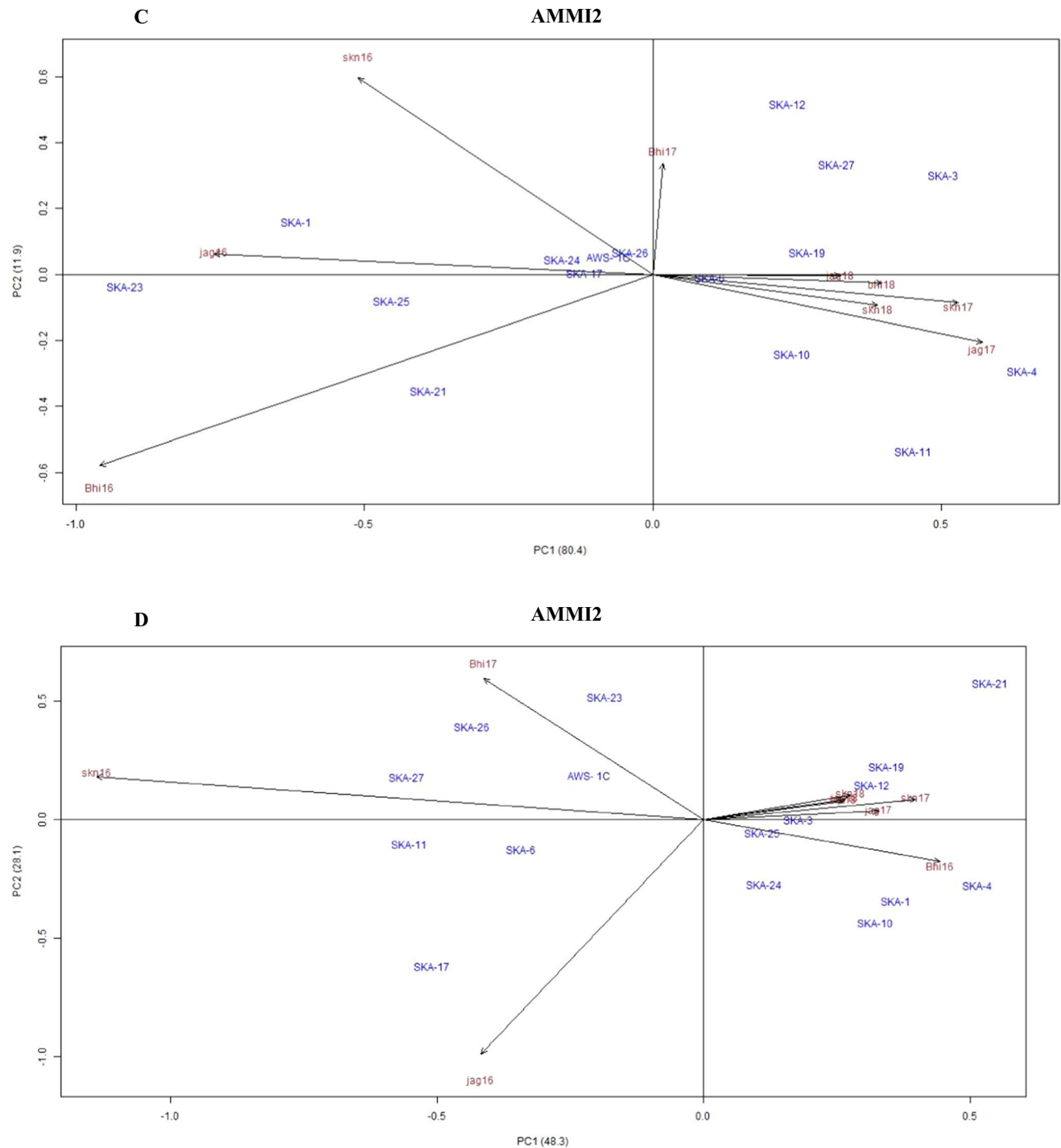
When environments were assessed independently, AMMI1 also depicted the stability of genotypes in multiple environment for mean dry root yield and total root alkaloid content (Fig. 1A, B respectively). Environment and genotypes with the same IPCA1 sign interact positively, while those with different signs interact negatively. AMMI1 biplot graph for dry root yield showed that genotypes with negative IPCA1 viz., SKA-26, AWS-1, SKA-17, SKA-24, SKA-25, SKA-1, SKA-21 and SKA-23 interacted favourably with the environment Skn16, Jag16 and Bhi16. The genotypes with positive IPCA1 namely, SKA-3, SKA-4, SKA-6, SKA-12, SKA-19, SKA-10, SKA-27 and SKA-11 showed positive



**Fig. 1** AMMI biplot showing AMMI1 for **A** dry root yield and **B** total root alkaloid content. AMMI biplot showing AMMI2 for **C** dry root yield and **D** total root alkaloid content

interaction with Skn17, Jag17, Jag18, Skn18 and Bhi18 for dry root yield (Fig. 1A). From AMMI1 biplot for total root alkaloid content; SKA-17, SKA-23, AWS-1, SKA-6, SKA-26, SKA-27 and SKA-11 with negative IPCA1 score had

positive interaction with environments Bhi17, Jag16 and Skn16. Genotypes positive for IPCA1 namely, SKA-24, SKA-3, SKA-4, SKA-21, SKA-1, SKA-19, SKA-10, SKA-12 and SKA-25 interacted positively with Bhi16, Skn17,



**Fig. 1** (continued)

Jag17, Skn18, Jag18 and Bhi18 (Fig. 1B). Additionally, it is also established that genotypes close to the  $x$ -axis and on the right side of the  $y$ -axis are stable and more efficient, whereas genotypes distant from the  $x$ -axis and on the left side of the  $y$ -axis are unstable and low-yielding (Crossa et al. 1990). Accordingly, genotype SKA-11 exhibited greatest dry root

yield and quite stable followed by SKA-10 and SKA-27. The most stable genotype, SKA-19, had the highest dry root output per plant. Moreover; SKA-4, SKA-23, SKA-1, SKA-3, SKA-25 and SKA-21 were unstable across locations for dry root yield (Fig. 1A; Table 2). Similarly, for total root alkaloid, SKA-25 and SKA-12 was high in yield and quite

**Table 1** AMMI analysis of variance of dry root yield and total root alkaloid content for the Indian ginseng genotypes grown over 3 years and 3 locations

Sources of variation	Degree of freedom	Dry root yield				Total root alkaloid content							
		Sum of squares	Mean sum of squares	F value	Pr (> F)	% explained	% accumulated	Sum of squares	Mean sum of squares	F value	Pr (> F)	% explained	% accumulated
Environment (E)	8	20.74	2.60	42.07	3.112e-06***	35.31		14.30	1.79	60.98	6.196e-07***	27.59	
Rep(E)	9	0.56	0.07	2.42	0.01439*	0.96		0.27	0.03	0.69	0.7167	0.52	
Genotype (G)	15	14.62	0.98	38.13	2.2e-16***	24.89		9.18	0.62	14.41	2.2e-16***	17.72	
G×E interaction	120	19.36	0.17	6.31	2.2e-16***	32.96		22.35	0.19	4.39	3.001e16***	43.13	
IPCA1	22	15.57	0.71	27.68	0.00***	80.4		10.79	0.49	11.55	0.00***	48.3	48.3
IPCA2	20	2.30	0.12	4.50	0.00***	11.9		6.27	0.32	7.39	0.00***	28.1	76.3
IPCA3	18	1.08	0.06	2.33	0.01**	5.5		4.39	0.25	5.74	0.00***	19.6	96.0
IPCA4	16	0.25	0.02	0.60	0.88 <sup>NS</sup>	1.3		0.79	0.05	1.16	0.31 <sup>NS</sup>	3.5	99.5
IPCA5	14	0.11	0.008	0.29	0.995 <sup>NS</sup>	0.5		0.09	0.007	0.15	0.99 <sup>NS</sup>	0.4	99.9
IPCA6	12	0.07	0.006	0.21	0.998 <sup>NS</sup>	0.3		0.02	0.002	0.04	1.00 <sup>NS</sup>	0.1	100.0
IPCA7	10	0.006	0.0006	0.02	1.00 <sup>NS</sup>	0.0		0.003	0.001	0.01	1.00 <sup>NS</sup>	0.0	100.0
IPCA8	8	0.003	0.0003	0.01	1.00 <sup>NS</sup>	0.0		0.002	0.0002	0.00	1.00 <sup>NS</sup>	0.0	100.0
Residuals	135	3.46	0.03			5.89		5.73	0.05			11.06	

IPCA interaction principal component analysis axis; NS nonsignificant

\*\*\*: 0.001, \*\*: 0.01, \*: 0.05

**Table 2** Average dry root yield (g) and total root alkaloid content (%) of ashwagandha (Y) and other stability parameters: additive main effects and multiplicative interaction (AMMI) stability index (ASI), rankings of asi (RASI), rankings of mean dry root yield (RY), simultaneous selection index (SSI) and first and second interaction principal component axis (IPCA1 & IPCA2)

Genotype	Y	ASI	rASI	rY	SSI	IPCA1	IPCA2
<i>Dry root yield (g)</i>							
SKA-1	1.31	0.50	14	15	29	-0.62	0.16
SKA-3	1.44	0.41	13	10	23	0.51	0.30
SKA-4	1.58	0.52	15	7	22	0.65	-0.29
SKA-6	1.45	0.08	3	9	12	0.09	-0.01
SKA-10	1.785	0.196	6	4	10	0.25	-0.25
SKA-11	2.08	0.369	12	1	13	0.46	-0.54
SKA-12	1.54	0.198	7	8	15	0.24	0.52
SKA-17	1.376	0.09	4	13	17	-0.12	0.01
SKA-19	1.61	0.22	8	6	14	0.27	0.07
SKA-21	1.74	0.32	10	5	15	-0.39	-0.36
SKA-23	1.787	0.74	16	3	19	-0.92	-0.04
SKA-24	1.379	0.13	5	12	17	-0.16	0.05
SKA-25	1.26	0.363	11	16	27	-0.45	-0.08
SKA-26	1.37	0.04	1	14	15	-0.04	0.07
SKA-27	1.88	0.26	9	2	11	0.32	0.34
AWS-1	1.42	0.07	2	11	13	-0.08	0.06
<i>Total root alkaloid content (g)</i>							
SKA-1	1.37	0.20	8	11	19	0.36	-3.44
SKA-3	1.16	0.09	2	15	17	0.18	-7.97
SKA-4	1.32	0.26	12	13	25	0.52	-2.78
SKA-6	1.428	0.19	7	4	11	-0.35	-1.24
SKA-10	1.40	0.23	10	7	17	0.33	-4.36
SKA-11	1.80	0.27	13	1	14	-0.57	-1.04
SKA-12	1.42	0.17	5	6	11	0.32	1.48
SKA-17	1.21	0.304	15	14	29	-0.51	-6.19
SKA-19	1.378	0.21	9	10	19	0.35	2.26
SKA-21	1.39	0.309	16	8	24	0.54	5.80
SKA-23	1.36	0.18	6	12	18	-0.19	5.18
SKA-24	1.09	0.16	4	16	20	0.12	-2.72
SKA-25	1.425	0.06	1	5	6	0.11	-5.68
SKA-26	1.50	0.24	11	3	14	-0.44	3.94
SKA-27	1.77	0.28	14	2	16	-0.56	1.80
AWS-1	1.38	0.13	3	9	12	-0.22	1.87

stable across the environment. SKA-23 and AWS-1 was though low in yield but significantly stable while, genotypes SKA-21, SKA-4, SKA-11, SKA-27, SKA-17 and SKA-26 were most unstable for total root alkaloid content (Fig. 1B; Table 2).

Stable genotypes are considered desirable when they have high mean performance and are situated near to the ideal genotype in GGE biplot (Yan and Tinker 2006). AMMI2 biplot depicted genotypes SKA-17, SKA-26, AWS-1, and SKA-24 as the most stable for dry root yield. SKA-6, however, was the most desired genotype due to its great stability and superior yield. Genotypes, SKA-1 and SKA-23 showed specific adaptability to environment Jag16 and SKA-25, SKA-21 to Bhi16 whereas, SKA-4 and SKA-11 to Jag17, Skn17, Skn18, Bhi18 and Jag18 for dry root yield

(Fig. 1C). SKA-25 was shown to be the most productive genotype for yield and stability in AMMI2 investigation for total root alkaloid content. SKA-12 and SKA-19 were though high yielding, but showed specific adaptability to few environment viz., Skn17, Jag17, Skn18, Jag18 and Bhi18 (Fig. 1D). Genotypes SKA-10, SKA-1 and SKA-4 showed specific adaptability to Bhi16; AWS-1, SKA-23 and SKA-26 to Bhi17; and SKA-27 and SKA-11 to Skn16 for total root alkaloid. The total root alkaloid content of the SKA-21 and SKA-6 genotypes demonstrated inadequate general and specific adaptability to all examined environment (Fig. 1D).

Environment with IPCA1 scores close to or equal to zero have little effect on interactions, but they have a big impact on the stability of genotypes (Oliveira et al. 2009). AMMI1 biplot showed environment, Jag18, Bhi18 and

**Table 3** Environmental means, IPCA1 and IPCA2 scores and relative rankings of first three genotypes based on dry root yield (g) and total root alkaloid content (%)

Environment	Mean		IPCA1	IPCA2	First	Second	Third
<i>Dry root yield (g)</i>							
Skn16	1.31	1.51	-0.52	0.67	SKA-23	SKA-27	SKA-12
Skn17	1.73		0.53	-0.09	SKA-11	SKA-27	SKA-4
Skn18	1.493		0.39	-0.11	SKA-11	SKA-27	SKA-10
Jag16	1.68	1.64	-0.77	0.07	SKA-23	SKA-21	SKA-1
Jag17	1.74		0.58	-0.23	SKA-11	SKA-27	SKA-10
Jag18	1.496		0.33	-0.00	SKA-11	SKA-27	SKA-10
Bhi16	2.05	1.54	-0.96	-0.65	SKA-23	SKA-21	SKA-11
Bhi17	1.05		0.02	0.38	SKA-10	SKA-27	SKA-19
Bhi18	1.53		0.40	-0.03	SKA-11	SKA-27	SKA-10
Mean	1.57	1.57					
Range	1.05–2.05	1.51–1.64					
<i>Total root alkaloid content (%)</i>							
Skn16	1.55	1.49	-1.14	2.01	SKA-27	SKA-11	SKA-6
Skn17	1.466		0.40	9.51	SKA-21	SKA-19	SKA-27
Skn18	1.468		0.28	1.14	SKA-11	SKA-27	SKA-21
Jag16	1.54	1.50	-0.42	-1.10	SKA-17	SKA-11	SKA-27
Jag17	1.477		0.33	4.00	SKA-21	SKA-10	SKA-19
Jag18	1.473		0.27	9.28	SKA-11	SKA-27	SKA-21
Bhi16	0.79	1.21	0.45	-1.95	SKA-11	SKA-10	SKA-25
Bhi17	1.35		-0.42	6.64	SKA-27	SKA-11	SKA-23
Bhi18	1.478		0.27	8.78	SKA-11	SKA-27	SKA-21
Mean	1.40	1.40					
Range	0.79–1.55	1.21–1.50					

IPCA1 & IPCA2 interaction principal component analysis axis 1 and 2 respectively

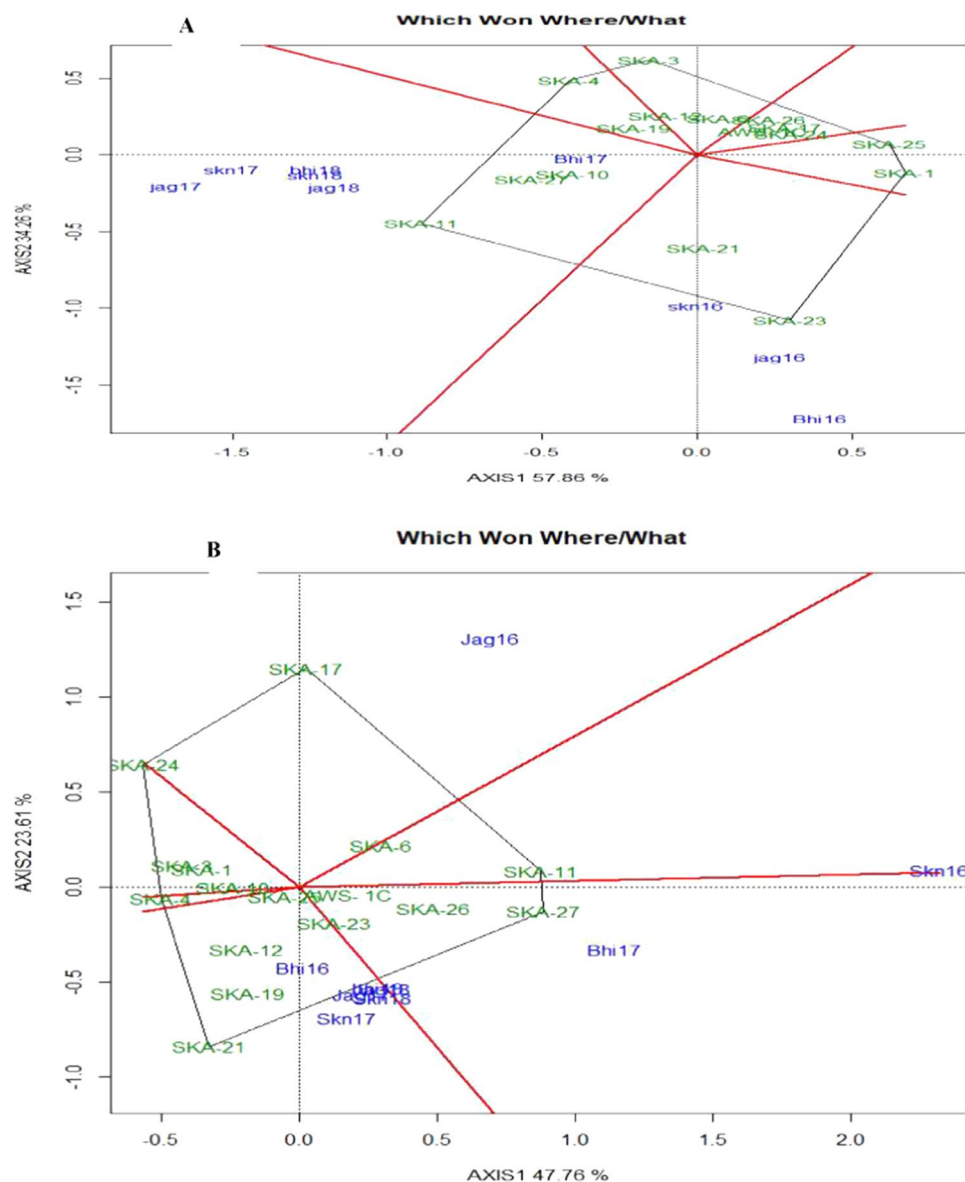
Skn18 to contribute high; Skn17, Jag17 and Skn16 as moderate and Bhi17, Jag16 and Bhi16 to contribute less towards stability of genotypes for dry root yield (Fig. 1A). Environments Jag18, Bhi18, and Skn18 contributed significantly to the stability of genotypes, while environments Bhi16 and Skn16 contributed substantially less to stability and more to GE interaction, according to AMMI1 study for total root alkaloid content (Fig. 1B). AMMI2 biplot graphs in Fig. 1C, D showed environment scores for dry root yield and total root alkaloid. In AMMI2, environments with low IPCA1 and IPCA2 values that were located close to the origin contributed significantly to genotype stability but little to GE interaction. Bhi16, Jag16, Skn16, Jag17 and Skn17 environment placed far from the biplot origin contributed less towards the stability while environment Jag18, Bhi18 and Skn18 contributed moderately towards the stability for dry root yield. While, the environment Bhi17 contributed solely towards the stability for dry root yield (Fig. 1C). For total root alkaloid content, Skn16, Jag16 and Bhi17 accorded comparatively high to the GE interaction and hence less towards the stability, environment Jag17, Skn17 and Bhi16 contributed moderately and Jag18, Bhi18 and Skn18 more for stability for total root alkaloid content (Fig. 1D).

### GGE biplot analysis and discriminating ability and representativeness of environments

In the GGE biplot analysis, the PC1 and PC2 explained 92.12% and 71.37% of the total GEI for dry root yield and total root alkaloid content, respectively. The which-won-where biplots from the present study for dry root yield and total root alkaloid content are presented in Fig. 2A and B, respectively. Two mega environments were reported for dry root yield (Fig. 3). The first mega environment included Skn16, Jag16 and Bhi16 while the second included Bhi17, Bhi18, Skn18, Jag18, Skn17 and Jag17 (Figs. 2A, 3). Moreover, genotypes, SKA-21 and SKA-23 were included in first mega environment while, SKA-10, SKA-27 and SKA-11 in the second mega environment (Fig. 2A). For the overall root alkaloid content, four mega-environments were recorded (Figs. 2B, 3). The first mega-environment comprised Jag16, second Skn16, third Bhi17 and fourth included Bhi16, Bhi18, Jag17, Jag18, Skn18 and Skn17 (Figs. 2B, 3). The mega-environment first, second, third and fourth included SKA-17; SKA-11; AWS-1, SKA-26, SKA-27 and SKA-25, SKA-12, SKA-19, SKA-21 genotypes, respectively (Fig. 2B). From which-won-where biplots, total root alkaloid content biplot may be considered as comparatively



**Fig. 2** GGE biplot showing which-won-where/what analysis for **A** dry root yield and **B** total root alkaloid content



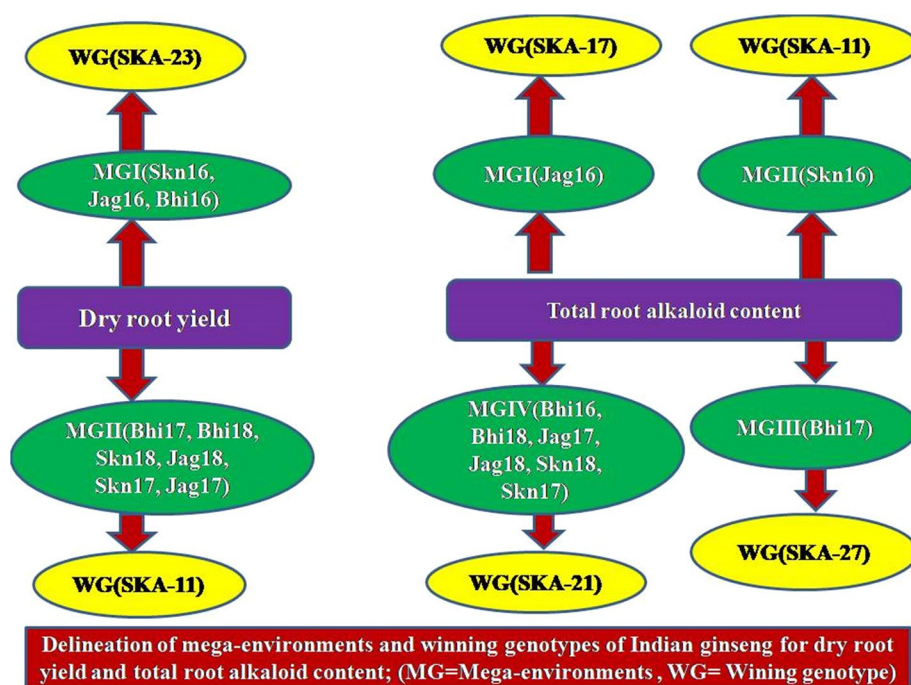
more informative since it could discriminate environment more efficiently. For dry root yield, the hexagon consisted six genotypes at the vertices and mega-environment first had SKA-23 as the winning genotype and second mega-environment had SKA-11 as winning genotype (Fig. 3). In the present study, genotype SKA-23 was found most suitable for all the three different locations in the cropping season 2016–2017, whereas for 2017–2018 and 2018–2019; SKA-11 was reported to be most efficient. Six genotypes were present at each vertex of the polygon representing the total root alkaloid content. Among four mega-environments identified, SKA-17 was winning in first, SKA-11 in second, SKA-27 in the third and SKA-21 in the fourth mega-environment (Fig. 3). In the present study, Bhi17, Bhi18, Skn17, Skn18, Jag17 and Jag18 were highly correlated environment and among these, Bhi17 contributed more towards the stability

for dry root yield (Fig. 4A). The SKA-10 and SKA-27 were desirable in these environments. Similarly, environments Skn16, Jag16 and Bhi16 showed high correlation among them and SKA-23 was found to be superior in these environments for dry root yield (Fig. 4A). For total root alkaloid content Bhi16, Jag17, Jag18, Skn18, Bhi18 and Skn17 were highly correlated and SKA-23 was most efficient genotype for these environment while, SKA-11 and SKA-27 were better genotypes in Skn16 and Bhi17 environments (Fig. 4B).

## Discussion

AMMI and GGE biplots are effective methods for analysing MET data and deciphering intricate GEI interactions (Yan and Tinker 2006). It may clearly graphically illustrate the

**Fig. 3** Delineation of mega-environments and winning genotypes of Indian ginseng for dry root yield and total root alkaloid content. *MG* mega-environment; *WG* winning genotype



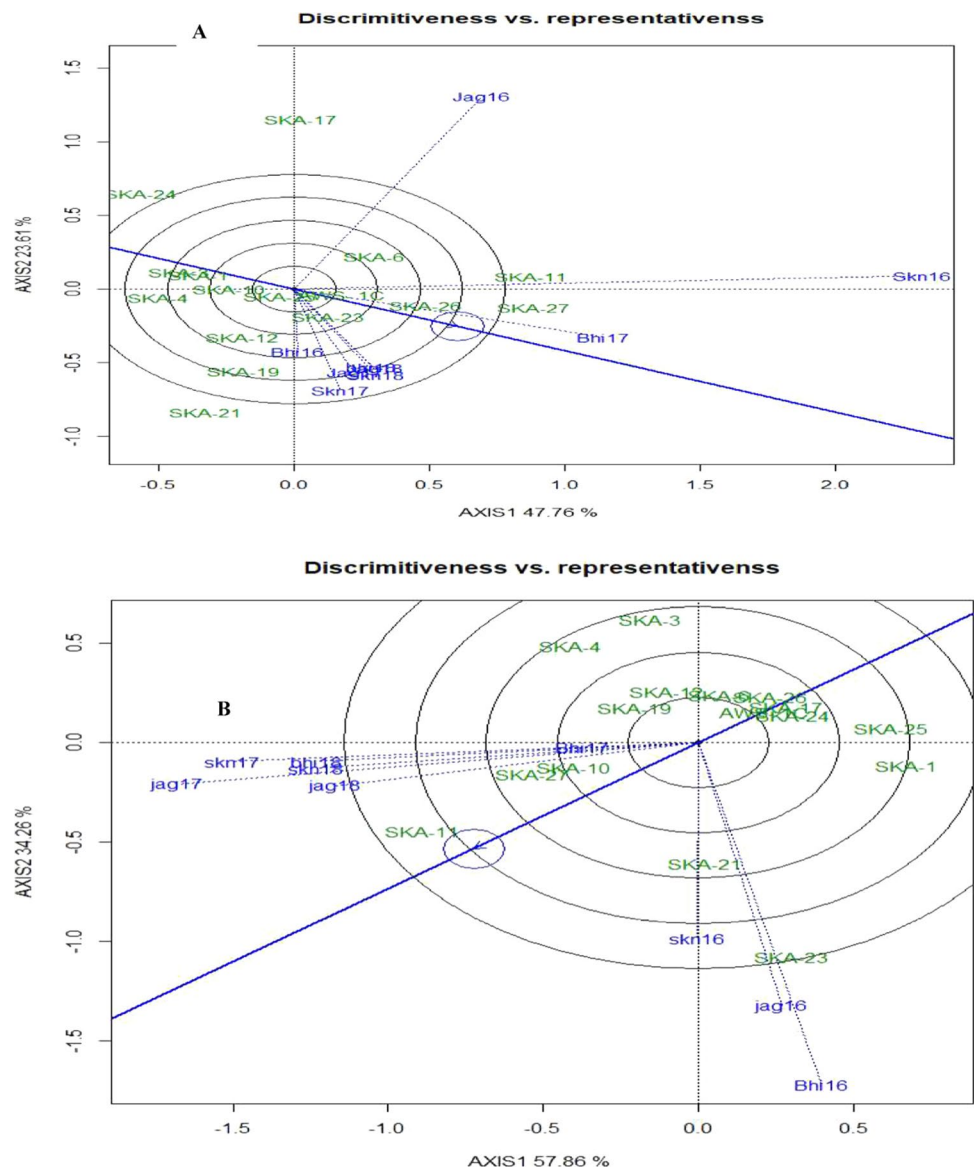
interaction pattern and identify mega-environments between the testing sites (Yan et al. 2007). However, the multi-location data in Indian ginseng have not yet been analysed using these techniques. In the present investigation, 16 genotypes of Indian ginseng were assessed by AMMI and GGE biplot models and SSI statistics for simultaneous selection of genotypes with high stability and yield in terms of dry root yield and total alkaloid content. Despite the fact that there is enough variation in medicinal plants, it has been stated that the exploitation of their genetic potential is still in its early phases (Canter et al. 2005). The results revealed that total alkaloid content, a secondary metabolite was significantly higher in unfavourable years (2017–2018 and 2018–2019) compared to favourable year (2016–2017) characterized on the basis of rainfall received. Due to stress-induced reductions in biomass production and subsequent biosynthesis of secondary metabolites, water stress increases accumulation of secondary metabolites (Selmar and Kleinwachter 2013; Ninemets 2015; Van De Velde et al. 2015; Shahriari et al. 2018). The current findings reveal that Indian ginseng dry root yield as well as root alkaloid content are impacted by the environment.

For dry root yield and root alkaloid content, AMMI analysis of variance revealed a significant influence of environment and G–E interaction in comparison to genotype. Similar results were also observed in triticale (Kendal et al. 2019), groundnut (Oliveira and Godoy 2006; Ajay et al. 2019), yellow passion fruit (Oliveira et al. 2009), wheat (Neisse et al. 2018) and ashwagandha (Kumar et al. 2020b). Since G and GEI rank genotypes, reducing or minimising the impact of environmental major

effects is sought (Gauch 1992). Indian ginseng breeders should therefore, take these findings into account during developing varieties for target locations. In the present investigation, AMMI2 biplot showed genotype, SKA-6 to be highly stable for dry root yield. Similarly, genotype, SKA-25 was widely adapted for total root alkaloid content, but it showed special adaptation to environment, Bhi16 for dry root yield. As a result, different responses in terms of dry root production and total root alkaloid content were found, which was consistent with the earlier findings of Shahriari et al.(2018).

The equality line of “which-won-where” GGE biplot eases the graphical comparison of distance between genotypes and environment and report representative environment and their capacity to discriminate (Lin and Binns 1988). Finding mega-environments makes it possible to locate areas that can be removed without losing crucial genotype-related information (Akinwale et al. 2014). In the present study, dry root yield was affected mostly by rainfall pattern and studied environments were divided into two meaningful mega-environments on the basis of amount of precipitation received during the experimental years. Environments Skn16, Jag16 and Bhi16 that received high rainfall were grouped in one mega-environment while remaining environments with low rainfall were grouped in the separate mega-environment. The mean comparison of environment showed that highest average dry root yield was obtained from Jagudan followed by Bhiloda and S. K. Nagar. The primary element contributing to the high dry root production in Bhiloda and Jagudan is probably the favourable edaphic conditions for greater root proliferation and their general

**Fig. 4** Discriminateness and representativeness of environments for **A** dry root yield and **B** total root alkaloid content



growth along with development inside the soil throughout the crop establishment stage.

The information on mega-environments enable breeders to identify discriminating and representative test environment for identifying broadly or specifically adapted genotypes (Akinwale et al. 2014). GGE biplot analysis for dry root yield showed that trait was discriminated by the amount of rainfall received in particular year. So, significant effects of environments were reported in correspondence to previous findings on medicinal plants (Mathur et al. 2003; Cavaliere 2009; Shahriari et al. 2018). GGE biplot revealed that Bhi17 and Bhi16 were the most discriminating and representative environment for dry root yield and total root alkaloid content, respectively. The amount of rainfall during cropping period was discriminating for specifically adapted genotypes for dry root yield but it was not representative

except Bhi17. The results were in agreement with previous reports on mucilage content in *Plantago* that showed variable responses to environmental conditions (Shahriari et al. 2018).

AMMI model is effective as it contributes large portion to GEI sum of squares and separate the main and interaction effects. The optimum cultivars for mega-environments and stable genotypes across environments can be found using the GGE biplot model (Roostaei et al. 2014). The results showed that AMMI and GGE biplot models had similar results for dry root yield but contrasting results for total root alkaloid content in view of wider adaptation. However, similar results were obtained for specific adaptation of genotypes to environment from both the models. In the present study, similar results were obtained for environmental contribution towards the genotypes from AMMI1 and AMMI2 analysis for dry

root yield and total root alkaloid content except environment, Bhi17. In the GGE biplot analysis, Bhi17, Bhi18, Skn17, Skn18, Jag17 and Jag18 were highly correlated and formed single cluster while Skn16, Jag16 and Bhi16 formed different cluster for dry root yield. The cluster formed for environmental effect towards the genotypes differed for total root alkaloid content. For dry root yield of the top-ranked genotypes in all environment, SSI statistic's findings were consistent with those of the AMMI1, AMMI2, and GGE biplot models. The SSI data for total root alkaloid content agreed completely with those of the genotype rankings from AMMI1 and AMMI2, but not with GGE biplot model.

## Conclusions

For dry root yield and total root alkaloid content, respectively, Bhi17 and Bhi16 were said to be the most discriminating and representative environments. The genotypes SKA-11 and SKA-27 were found to be the most efficient for both dry root yield and total root alkaloid content according to the stability analysis using GGE biplot and AMMI. These are the genotypes that are recommended across environments to increase Indian ginseng's alkaloid content and root yield. However, location-specific adaptation of genotypes reported in present study clearly suggested the need of location-specific breeding. To consider them for release, efforts must be made to identify genotypes adapted to particular area across years and locations.

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**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors declare no conflicts of interest.

**Human and animal rights** This article does not contain any studies with human or animal subjects.

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