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## Saudi Journal of Biological Sciences

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Original article

## Early selective strategies for higher yielding bio-economic Indian ginseng based on genotypic study through metabolic and molecular markers

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## ARTICLE INFO

## Article history:

Received 24 August 2021

Revised 11 January 2022

Accepted 13 January 2022

Available online 20 January 2022

## Keywords:

Withanolides

Genetic diversity &amp; gain

Path analysis

Ginseng

Phenotypes selection

## ABSTRACT

Applying biotechnological tools to the selection of higher-yielding bioeconomic crops is a promising and remarkable means of reducing the burden on production on a global scale. In the present study, 25 germplasms of Indian ginseng (*Withania somnifera* (L.) Dunal) were examined for their genetic diversity by using morphological, biochemical, and molecular markers for twenty plant growth traits. The properties of plant growth differed significantly in the maximum genotypes of Indian ginseng, the markers of randomly amplified polymorphic DNA (RAPD), and inter simple sequence repeat (ISSR) showed considerable diversity between the genotypes. The combined unweighted pair group technique with arithmetic mean (UPGMA) dendrogram of morphological, biochemical, and molecular markers grouped all 25 genotypes into two main clusters at 0.61 coefficient value. In addition to this, secondary metabolite profiling by high-performance liquid chromatography (HPLC), there were high variations for withanolide B (WL-B), withanoside-V (WS-V), wedelolactone (WDL), withanoside-IV (WS-IV), and withaferin A (WF-A) content between different genotypes. For the total alkaloid and withanolide concentration in the roots and leaves, high heritability with an increased genetic gain was observed, indicating that selection based on these traits could be an effective method in breeding programs. Furthermore, the path coefficient analysis showed a direct positive impact of the total root fiber, WL-B (leaves), WF-A (leaves), WS-IV (roots), WDL (roots), and the total alkaloid content on the dry root yield. High content of WDL, a high-quality bioactive withanolide, was also described for the first time in the genotype UWS23. These properties can further be exploited to improve the dry root yield in *W. somnifera* genotypes. The outcomes of the present study also provide an essential foundation for the selection of high-yielding bioeconomic varieties that could be utilized to improve Ashwagandha breeding programs.

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**Abbreviations:** RAPD, randomly amplified polymorphic DNA; ISSR, inter simple sequence repeat; UPGMA, unweighted pair group technique with arithmetic mean; HPLC, high performance liquid chromatography; WL, withanolide; WS, withanoside; WDL, wedelolactone; WF, withaferin; PIC, polymorphism information content; EMR, effective multiple ratio.

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Peer review under responsibility of King Saud University.



## 1. Introduction

Due to the increasing population pressure, the demand and the consumption of food are increasing rapidly worldwide. This requires increasing food production from agribusiness while balancing the production and consumption of nutritious foods but remains a difficulty for all nations (Zia-Ul-Haq et al., 2013; Kiran et al., 2020; Priyadarshini and Abhilash, 2021). From the point of view of agricultural practices and food security, the grading of plant species according to their bio-economic value is increasingly recognized (Dangol et al., 2020; Kouhi et al., 2020; Nawaz et al., 2020).

Recently, the global demand for nutritious foods has mainly focused on immune boosting food ingredients. Several plant

<https://doi.org/10.1016/j.sjbs.2022.01.030>

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species with recognized medicinal properties have been attempted to increase their cultivation. However, the suitable candidate for plant species at the field level is being studied in part, particularly because of its high bioeconomic value properties. The Indian ginseng plant (*Withania somnifera* (L.) Dunal) is a high bioeconomic value plant and is one of the world's most valuable medicinal herb with diverse pharmacological properties (Jang et al., 2020).

It is a small woody herb of the Solanaceae family, popularly known as winter cherry, Indian ginseng, Ashwagandha, or Asgand (Bharti et al., 2016; Sangwan et al., 2007). The ginseng plant contains countless active ingredients that have miraculously treated human health disorders for ~4000 years and are considered an essential part of the Indian Ayurvedic, Siddha, and Unani medical systems (Chauhan et al., 2019; Singh and Kumar, 1998). Given its unprecedented therapeutic potential, the Indian pharmacopeia officially documents the plant since 1985 as a drug (Singh et al., 2011; Uddin et al., 2012), commonly used to treat various kinds of diseases such as rheumatism, arthritis, amnesia, syphilis, anxiety, and asthma (Rasool and Varalakshmi, 2006; Bharti et al., 2016).

The antimicrobial, antiepileptic, immunosuppressive, anti-inflammatory, hepatoprotective, antitumoral, cardiovascular, anti-hyperglycemic, antioxidant and neuroprotective activities of this plant are also very well documented (Fernando et al., 2013; Furmanowa et al., 2001; Gorelick et al., 2015; Jain et al., 2012; Konar et al., 2019; Tiwari et al., 2014). It has been reported that more than 12 alkaloids, 130 withanolides, and many sitoinoside compounds have been isolated from *withania* sp. in the last two decades (Girme et al., 2020; Maurya, 2017; Mirjalili et al., 2009). Its phytomedicine value has been attributed primarily to withanolides, structurally a group of naturally occurring C28 steroidal lactones built on an ergostane skeleton containing a 6C lactone ring formed by oxidation of C22 and C26 (Jain et al., 2012; Mirjalili et al., 2009). Withanolide A (WL-A), withanolide B (WL-B), withanolide D (WL-D), withaferin A (WF-A) and withanoside (WS) I to VII are the important compounds in Ashwagandha (Jang et al., 2020; Matsuda et al., 2001). Wedelolactone (WDL) is another recently identified withanolide that has been reported to have hepatoprotective, anti-inflammatory, parkinsonism, and anti-tumor activities (Liu et al., 2016; Sarveswaran et al., 2016; Sharma et al., 2021; Svrlanska et al., 2020; Zhao et al., 2015).

India is the largest producer of Ashwagandha with more than thirty genetic varieties from the arid and semi-arid regions (Lal, 2015). For the international export market, it covers over 3000 million USD (Srivastava et al., 2018a). National production is estimated at 1500 t, while the annual demand is around 7000 t (Umadevi et al., 2012). Given its enormous economic and medicinal importance, this requires closing the production gap through a significant increase in ginseng cultivation, which largely depends on the local varieties (Yadav et al., 2007), therefore a detailed study of the important characteristics relevant to yield is enormously required using the available genotypes. In the past two decades, different genetic markers have been used either in combination or separately to identify taxon-specific plants from a morphological, biochemical and molecular point of view (Tigano et al., 2010; Dar et al., 2015). The different biotic and abiotic stress factors have led to a significant variation in the morphological characteristics and the biochemical composition between the species, so an accurate identification can be achieved if these markers are successfully coupled with the molecular markers (Kiran et al., 2010). The fact that physiological conditions and environmental pressures rarely affect DNA-based molecular markers are therefore used to identify different genotypes of the same species in order to distinguish genotypes of different genera by detecting DNA polymorphisms (Kaur et al., 2016; Kiran et al., 2010). Therefore, this study aimed to (i) assess the variability present in the local germ plasmas of Indian ginseng using morphomolecular markers and chemoprofiling of key bioactive compounds and (ii) examine the degree of genetic associations of various traits that make its yield.

## 2. Materials and methods

### 2.1. Plant material and experimental design

A total of 25 germplasms of Indian ginseng were obtained from various agricultural repositories with the accession numbers UWS-10, UWS-11, UWS-13, UWS-15, UWS-22, UWS-23, UWS-28, UWS-32, UWS-35, UWS-37, UWS-56, UWS-59, UWS-60, UWS-67, UWS-77, UWS-92, UWS-93, UWS-98, UWS-111, UWS-134 collected from germplasm collection of All India Coordinated Research Project (AICRP) on Medicinal & Aromatic Plants and Beetlevine, Udaipur, India, AWS2B-9 from AAU, Anand, India, HWS-08-14 from

**Table 1**  
Mean square for RBD in individual environment.

S. No.	Characters	Replication [2]	Genotype [24]	Error [48]
1	Plant height (PH)	13.72** cm	95.55** cm	0.47 cm
2	No. of primary branches/plant (NPBP)	0.36	1.21	0.69
3	Days to 75% flowering (D75F)	0.69	242.38**	0.98
4	Days to 75% maturity (D75M)	5.32**	62.77**	0.82
5	Root length (RL)	11.68** cm	23.45** cm	0.56 cm
6	Root diameter (RD)	5.32** cm	6.67** cm	0.82 cm
7	Dry root yield (DRY)	0.00 g	3.05** g	0.00 g
8	Total alkaloid content (TAC)	0.00** %	0.21** %	0.00 %
9	Total root starch content (TRSC)	3.88* %	30.78** %	0.84 %
10	Total root fibre content (TRFC)	1.16 %	81.28** %	2.28 %
11	Wedelolactone in roots (WDL R)	10.17** ng/μl	115.37** ng/μl	1.61 ng/μl
12	Wedelolactone in leaves (WDL L)	0.29 ng/μl	144247.09** ng/μl	5.42 ng/μl
13	Withanoside IV in roots (WIVR)	0.43 ng/μl	101412.13** ng/μl	1.01 ng/μl
14	Withanoside IV in leaves (WIV L)	0.00 ng/μl	6119753.33** ng/μl	0.92 ng/μl
15	Withanoside V in roots (WVR)	0.12 ng/μl	1196.30** ng/μl	0.91 ng/μl
16	Withanoside V in leaves (WVL)	0.35 ng/μl	2317.18** ng/μl	0.61 ng/μl
17	Withanolide B in roots (WBR)	0.12 ng/μl	1315.75** ng/μl	0.75 ng/μl
18	Withanolide B in leaves (WBL)	1.71 ng/μl	26057.88** ng/μl	1.35 ng/μl
19	Withaferin A in roots (WFAR)	1.85 ng/μl	18945.38** ng/μl	0.88 ng/μl
20	Withaferin A in leaves (WFAL)	0.05 ng/μl	147655.24** ng/μl	1.00 ng/μl

\* Significant at 5%.

CCSHAU, Hisar, India, and RVA-100, JA-20, JA-134 from RVSKVV, Mandsaur, India. The genotypes were grown in triplicate for two consecutive years (2018–19, 2019-20) in the Kharif session with randomized block design patterns, the experimental plot size (4 × 3 m<sup>2</sup>/genotype) was determined on the Instructional Farm, Rajasthan College of Agriculture, MPUAT, Udaipur (24035N, 70042E), Rajasthan (India). In both years the seeds were sown in the 3<sup>rd</sup> week of July and the plant material was harvested in the 2<sup>nd</sup> week of October. Standard cultural practices were followed to raise the harvest (Patra et al., 2001). Random samples of 10 plants were selected from each replication to record variations in key morphological traits, namely plant height (PH), primary branches per plant (NPBP), days to 75% flowering (D75F), days to 75% maturity (D75M.), Dry root yield (DRY), root length (RL) and root diameter (RD) related to the commercial value of the crop. Root and leaf samples were harvested during harvest time, oven-dried and stored at 40 °C in order to quantify the content of alkaloids, starch, fiber and withanolides.

### 2.2. Quantitative estimation of alkaloid, starch, and fiber and withanolides

The total alkaloid, total starch and total fiber content was estimated in dried root samples as previously described methods (Hodge and Hofreiter, 1962; Maynard, 1970). The extraction of withanolides was carried out as described earlier by Jain et al. (2011) in dried Leaf and root samples. Quantitative determination of various withanolides, including WF-A, WL-B, WS-IV, WS-V and WDL content, were analyzed by chromatogram obtained through HPLC-DAD (Agilent 1290, Germany) in both tissues of each genotype (Jain et al., 2011). The concentration of withanolide was calculated using the regression equations derived from the standard curve for each withanolide in leaf and root extracts of all genotypes (Figure S1-S2).

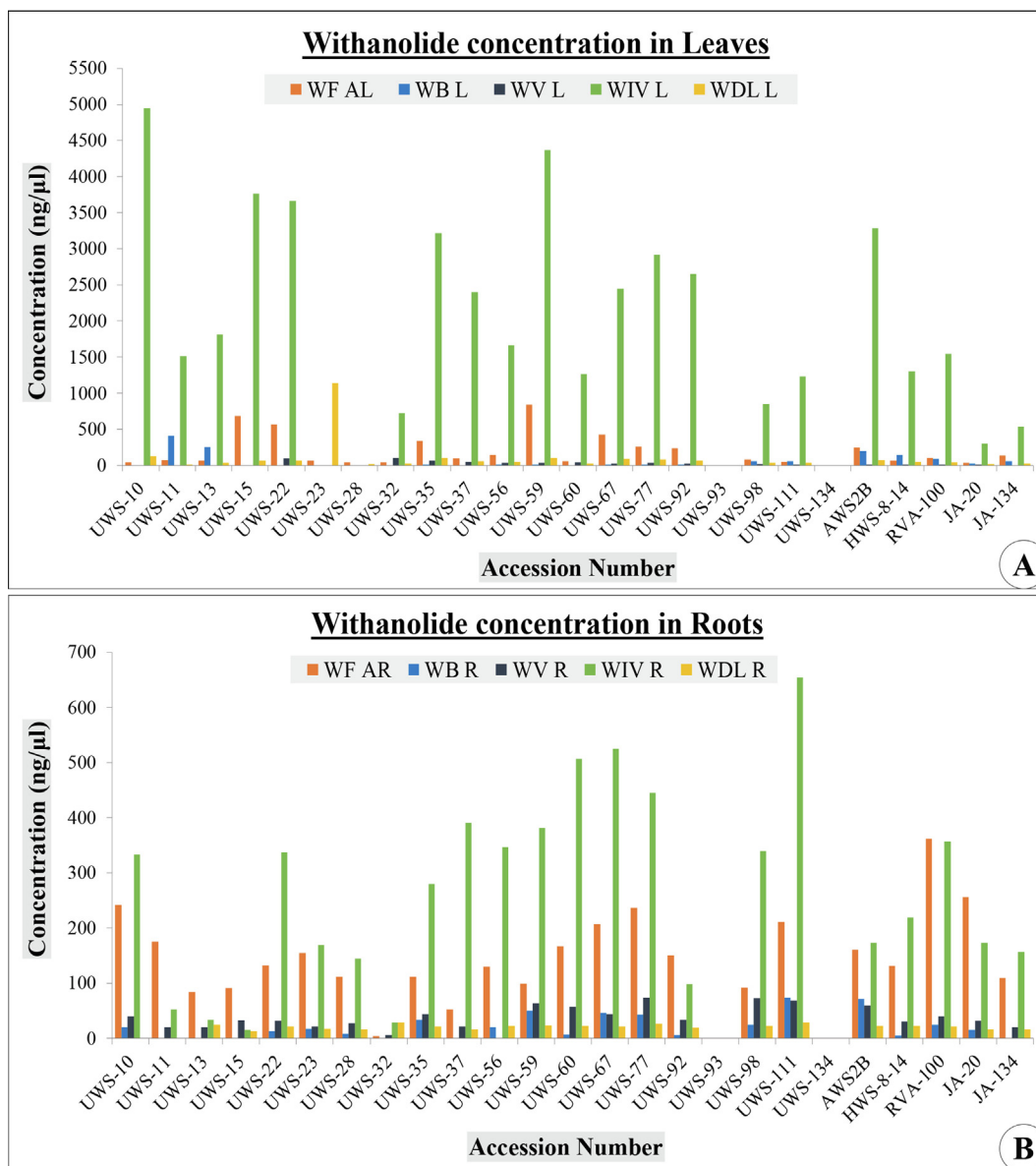


Fig. 1. Histogram representing the concentration (ng/μl) of wedelolactone (WDL L), withanoside IV (WIV L), withanoside V (WVL), withanolide B (WBL) and withaferin A (WFAL) in (A) leaves, and (B) roots of 25 genotypes of Indian ginseng.

2.3. Amplification of genomic DNA with RAPD and ISSR markers

Total genomic DNA was isolated from young leaves of all accessions using a standard protocol (Doyle, 1990) and its amplification was carried out using 40 primers corresponding to the OPA, OPD and OPB (Operon Technologies Inc.) series of RAPD and UBC series of ISSR markers belong as per previously reported method (Goyal et al., 2015). The PCR amplification was carried out in a 25 µL reac-

tion containing 200 µM dNTP mix, 1.5 mM MgCl<sub>2</sub>, 1 U Taq polymerase, 1X reaction buffer, 0.5 µM primer, 50 ng genomic DNA and milliQ grade water. The amplification was carried out in an Eppendorf Master Cycler (Germany) with programmed reaction conditions as initial pre-denaturation at 94 °C for 4 min, followed by 44 cycles of denaturation at 94 °C for 1 min, annealing at 32–52 °C for 1 min (as according to Table S4) and extension at 72 °C for 2 min. A final extension was carried out for 10 min at 72 °C.

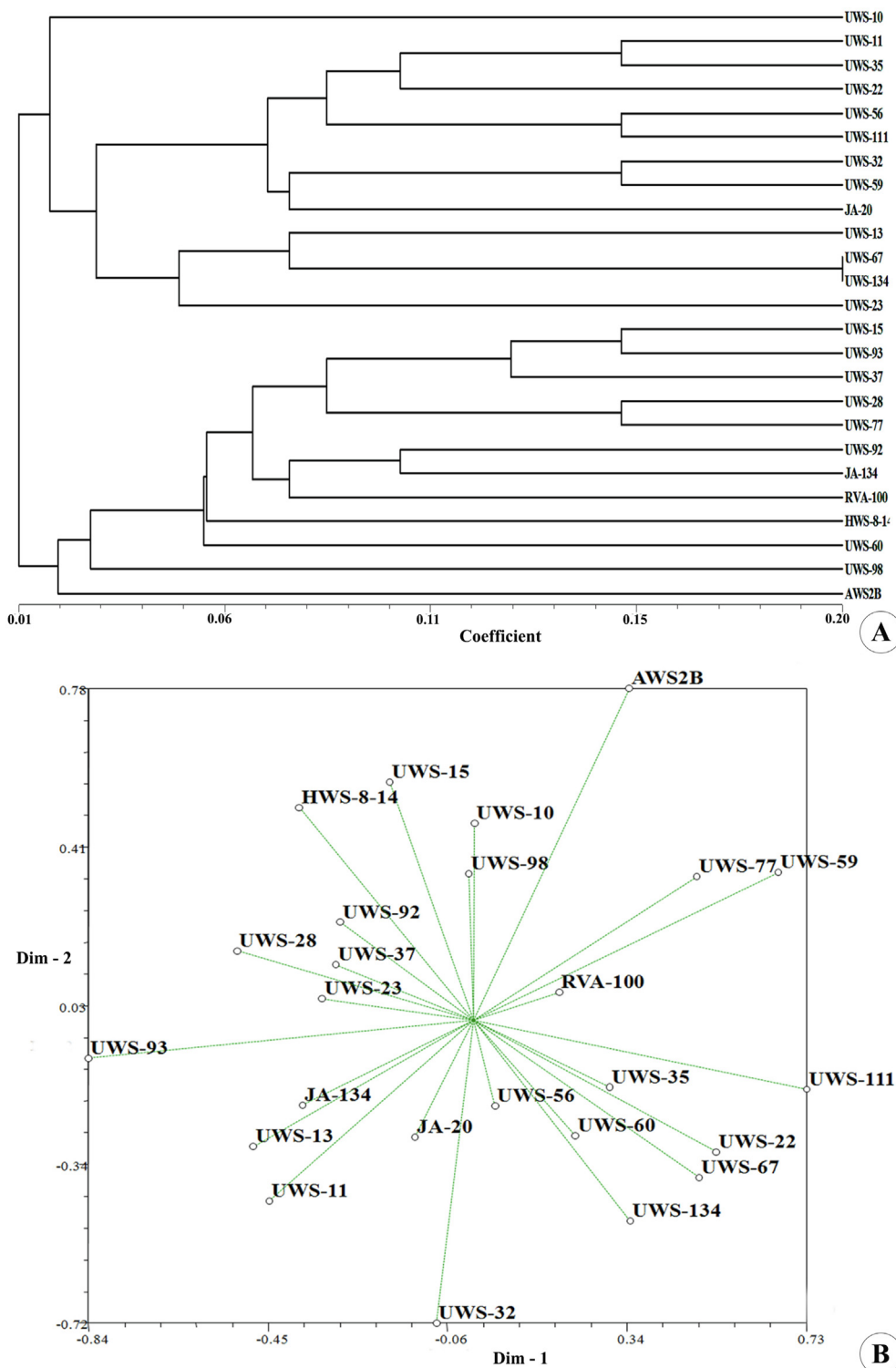


Fig. 2. (A) UPGMA dendrogram and (B) 2-D plot generated on the basis of morphological variations among 25 genotypes of Indian ginseng.

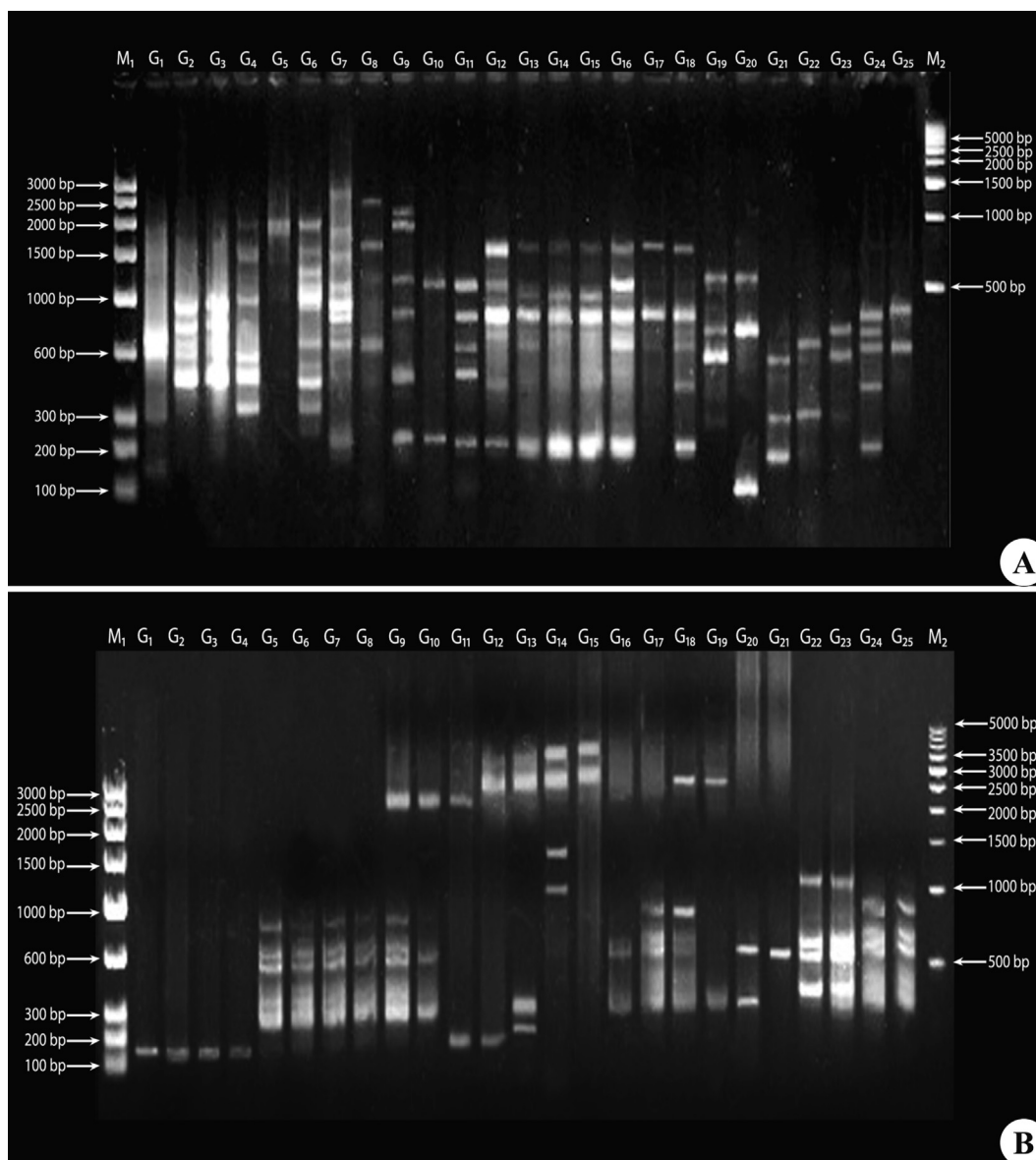
with a holding temperature of 4 °C. The amplification products were separated by electrophoresis on 1.2% (w/v) agarose gel, and stained with ethidium bromide (EtBr). The gel was photographed with a gel documentation system (Alpha DigiDoc, Germany).

#### 2.4. Path and data analysis

Analysis of variance (ANOVA) was performed on the pooled 2-year mean data for all 20 characteristics (PH, NPBP, D75F, D75M, RL, RD, DRY, TAC, TRSC, TRFC, WDL-R, WDL-L, WIV.) -R, WIV-L, WV-R, WV-L, WB-R, WB-L, WFA-R and WFA-L) using the 25 genotypes according to our previously reported method (Kaur et al., 2016) from MS Excel and NTSYS-pc 2.1 (Rohlf, 2004).

Amplified bands generated from RAPD and ISSR-PCR amplification were scored based on the presence (1) or absence (0) of bands for each primer and used to compute an SM similarity matrix using

NTSYS-pc Version 2.1 (Rohlf, 2004) to be calculated. Cluster analysis was performed on both morphological and molecular data and the similarity matrices were compiled for all accession pairs using SM similarity coefficients, using SIMQUAL, then cluster analysis using the unweighted pair group method with mean arithmetic analysis (UPGMA) and Dendrograms carried out were created with the SAHN program. A PCA was also performed using the same software to identify the genetic association between the genotypes. The Polymorphism Information Content (PIC), the effective multiple ratio (EMR), the resolving power (Rp) and the marker index (MI) were also calculated from the data of the RAPD and ISSR-PCR (Kaur et al., 2016). Furthermore, multivariate correlation, genetic association and path coefficient analyzes were carried out on the pooled mean value data according to the method described by (Dewey and Lu, 1959). The significance of the correlation was also tested according to the method of (Fisher and Yates, 1938).



**Fig. 3.** Representative Agarose gel electrophoresis of 25 genotypes of Indian ginseng showing amplification pattern with (A) RAPD (OPB-05) and (B) ISSR (UBC-813) markers. Lanes: M1&M2 DNA ladder; G1– G25 Different genotypes of Indian ginseng. (G1 - UWS 10; G2 - UWS 11; G3 - UWS 13; G4 - UWS 15; G5 - UWS 22; G6 - UWS 23; G7 - UWS 28; G8 - UWS 32; G9 - UWS 35; G10 - UWS 37; G11 - UWS 56; G12 - UWS 59; G13 - UWS 60; G14 - UWS 67; G15 - UWS 77; G16 - UWS 92; G17 - UWS 93; G18 - UWS 98; G19 - UWS 111; G20 - UWS 134; G21 - RVA 100; G22 - AWS2B; G23 - HWS-8-14; G24 - JA-20 and G25 - JA-134).

### 3. Results

In the present study, twenty different traits were used to assess the plant growth performance of the 25 genotypes shown in Table 1. A comparative profile of the withanolide content in leaves and roots of all 25 genotypes showed a higher content of all withanolides except that of WDL in the leaves (Fig. 1). The results of 20 traits from 25 genotypes were analyzed by ANOVA and found

that all traits were significant at 1% and 5%. Levels of significance, with the exception of the number of primary branches per plant (NPBP). The mean values of 20 traits, which were calculated from the two-year pooled data of all genotypes, are given in Tables S1-S2. The longest roots (22.4 cm) were produced in the genotype HWS-8-14, while the maximum root yield (4.58 g) was achieved in UWS 134 and UWS 67, respectively. The total content of alka-

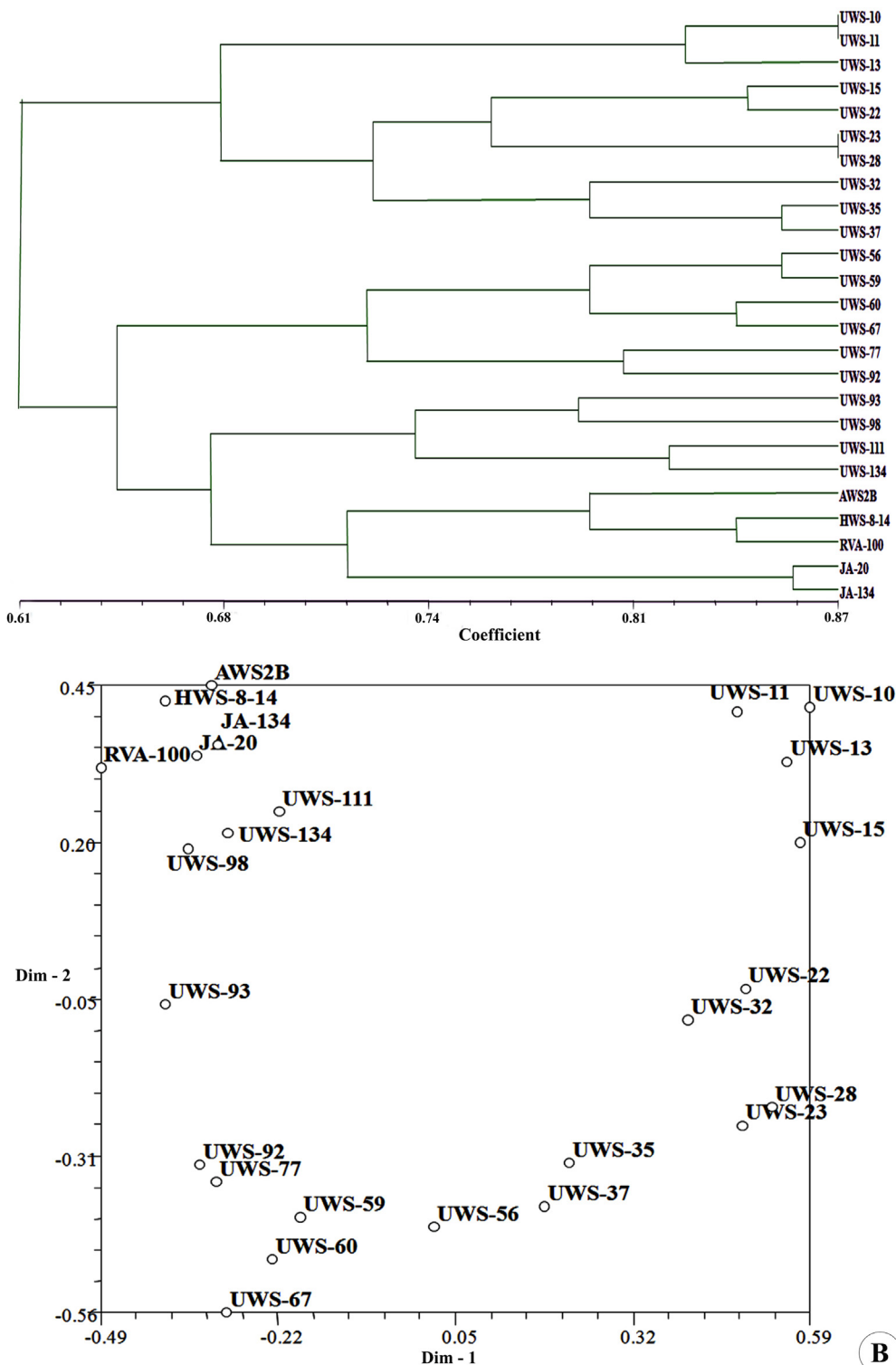


Fig. 4. (A) UPGMA dendrogram and (B) 2-D plot generated on the basis of combination of morphological and molecular variations among 25 genotypes of Indian ginseng.

loids, starch and fiber was maximal in the roots of UWS-59 (0.96%), UWS-59 & JA-134 (15.7%) and UWS-32 (36%), respectively.

### 3.1. Diversity analysis based on 20 morpho-biochemical traits/Morpho-biochemical diversity analysis

The pairwise similarity coefficient based on yield-related traits ranged from 0.01 to 0.20 for all genotypes with an average of 0.10. The correlation coefficient ( $r$ ) of 0.81 showed an excellent fit of the UPGMA cluster pattern. The UPGMA dendrogram based on phenotypic traits divided the 25 genotypes into two main groups, cluster I (13 genotypes) and II (12 genotypes), which were further subdivided into subclusters IA IB and IIA IIB (Fig. 2A). Genotypes UWS-67 and UWS-134 showed maximum similarity and could have evolved simultaneously (Fig. 2A). The genotypes showed a similar clustering pattern in the 2D plot as in the UPGMA dendrogram (Fig. 2B). PCA generated 19 major components (PCs), of which the first 16 PCs contributed 99.99% of the total variability. NPBP, D75F, DRY, total root strength, root fiber and WF-A content influenced all PCs (PC-I, PC-II and PC-III), while PH, D75M, RL, RD and total root alkaloid content both influenced the two PCs (Table S3).

### 3.2. Analysis of genetic diversity

The genetic diversity among the 25 genotypes was analyzed based on reproducibility and the polymorphism pattern generated by RAPD and ISSR primers (Fig. 3; Table S4). A total of 40 primers produced 334 bands, 292 of which were polymorphic while the remaining 42 bands were monomorphic. The maximal polymorphism was recorded with RAPD (89.91%) followed by ISSR (80.5%) primers. 5 RAPD and 2 ISSR primers generated unique bands in 6 (AWS 2B, UWS 56, UWS 59, UWS 32, UWS 37 and UWS 22) and 2 (RVA 100 and UWS 67) genotypes of ginseng (Table S5) and therefore can be used to develop a marker system for the rapid and accurate selection of specific high-yielding genotypes.

The Jaccards similarity index, calculated by rating the presence (1)/absence (0) of each amplicon, ranged from 0.29–0.85 and 0.21–0.82, with an average of 0.57 and 0.82, respectively. 0.51 for RAPD or ISSR primers. The UPGMA clustering pattern differed only in the number of genotypes grouped into clusters I and II and formed with RAPD (20 - I and 5 - II) and ISSR (15 I and 10 II) markers.

**Table 2**  
Variability parameters.

S. No.	Characters	GCV	PCV	$h^2$	GA	GG
1	Plant Height (cm)	14.24	14.34	98.54	11.51	29.12
2	Days to 75% flowering	10.26	10.33	98.79	18.37	21.02
3	Days to 75% maturity	2.96	3.02	96.18	9.18	5.98
4	Root length (cm)	16.89	17.49	93.22	5.49	33.59
5	Root Diameter (cm)	12.85	15.31	70.39	2.41	22.21
6	Dry Root Yield (g)	32.65	32.68	99.79	2.08	67.18
7	Total Alkaloid Content (%)	55.68	55.71	99.91	0.55	114.66
8	Total root starch content (%)	25.85	26.92	92.25	6.25	51.16
9	Total root fibre content (%)	18.58	19.37	92.03	10.14	36.72
10	Wedelolactone in roots (ng/ $\mu$ l)	31.15	31.81	95.92	12.42	62.85
11	Wedelolactone in leaves (ng/ $\mu$ l)	235.34	235.35	99.99	451.68	484.77
12	Withanoside IV in roots (ng/ $\mu$ l)	68.48	68.48	100.00	378.74	141.07
13	Withanoside IV in leaves (ng/ $\mu$ l)	76.13	76.13	100.00	2942.21	156.82
14	Withanoside V in roots (ng/ $\mu$ l)	53.67	53.73	99.77	41.07	110.44
15	Withanoside V in leaves (ng/ $\mu$ l)	116.76	116.81	99.92	57.22	240.44
16	Withanolide B in roots (ng/ $\mu$ l)	94.08	94.16	99.83	43.09	193.64
17	Withanolide B in leaves (ng/ $\mu$ l)	321.01	321.03	99.98	191.97	661.22
18	Withaferin A in roots (ng/ $\mu$ l)	54.99	55.00	99.99	163.69	113.27
19	Withaferin A in leaves (ng/ $\mu$ l)	117.33	117.33	100.00	457.01	241.70

### 3.3. Cumulative analysis of phenotypic and genetic diversity

Dendrogram based on combined polymorphism data, all 25 genotypes were grouped into 2 clusters (Fig. 4A). The pairwise similarity between the ginseng genotypes ranged from 0.61 to 0.87 with an average of 0.74. The correlation coefficient ( $r$ ) of 0.83 was considered a good match for the UPGMA cluster pattern. The 2-D representation generated from combined morphological, RAPD and ISSR data (Fig. 4B) supported the clustering pattern obtained from the UPGMA dendrogram (Fig. 4A). The combined analysis showed that UWS-11, UWS-13, and UWS-15 were more phylogenetically correlated. This can obscure the path for the introgression of high-yielding and resistant genes into commercial and agricultural strains (Adeniji et al., 2012). The superior accessions identified in this way were then submitted to NBPGR, New Delhi, the details of which are contained in the Additional File (Table S6).

### 3.4. Variability, correlation and path analysis

The estimated GCV, PCV, heritability, genetic advancement, and genetic gain varied widely for all measured traits examined (Table 2). Table 2 shows the coefficient of variation, which is a true relative measure of the variance between different characteristics. A wide range of PCV and GCV were observed for all traits, ranging from 3.02 to 235.35 and 2.96 to 235.34, respectively. The PCV was slightly higher than the corresponding GCV value, suggesting very little environmental impact in each trait and the variations could be mainly due to genotypic effects.

The heritability values may be used to estimate the expected genetic advance through selection. The heritability values was recorded to be very high (>92%) for all traits with the exception of RD (70.39%), which indicates the additive gene effect in the expression of these traits and further indicates that these traits are inheritable and are used in a further selection process be able.

The high genetic progress was observed in WS-IV, (2942.21), WF-A (457.01), WDL (451.68) levels of the leaves and WS-IV (378.74) levels of the roots. High heritability in connection with a high genetic gain for total alkaloid, WDL, WS-IV, WS-V, WL-B and WF-A contents in both the root and leaf tissue further confirmed that the variations are predominantly genetic, so these properties can be effectively used to select superior ginseng strains. A high genetic gain in WL B, WDL, WF-A and WS-V indicated that these traits are determined by additive genes.

**Table 3**  
Correlation matrix (P/G).

Traits	PH	NPBP	D75F	D75M	RL	RD	DRY	TAC	TRSC	TRFC	WDL R	WDL L	WIVR	WIVL	WVR	WVL	WBR	WBL	WFAR	WFAL
PH	1.00	0.03	0.16	-0.05	0.41*	0.47*	-0.26	-0.02	-0.28	-0.04	0.22	-0.02	-0.02	0.09	-0.13	0.24	-0.06	-0.21	-0.21	-0.09
NPBP	0.01	1.00	0.10	0.39	0.23	-0.10	-0.59**	-0.24	0.37	-0.93**	-0.06	-0.01	-0.46*	-0.19	0.16	-0.53**	-0.35	0.08	-0.25	-0.32
D75F	0.16	0.03	1.00	-0.19	-0.02	0.34	-0.33	0.14	-0.32	-0.04	-0.19	0.20	-0.18	0.52**	0.06	-0.14	0.23	0.14	0.22	0.12
D75M	-0.06	0.19	-0.19	1.00	-0.06	-0.12	0.19	0.34	-0.04	-0.10	0.23	-0.30	0.11	-0.09	0.21	0.08	-0.02	0.21	-0.14	-0.06
RL	0.39	0.14	-0.02	-0.06	1.00	-0.10	-0.38	0.11	0.12	-0.21	0.03	-0.02	-0.13	0.25	0.04	0.01	-0.17	0.13	-0.19	0.22
RD	0.40*	0.01	0.27	-0.10	-0.06	1.00	-0.06	-0.03	-0.35	0.17	0.08	-0.27	-0.27	0.10	-0.16	-0.03	-0.08	-0.02	0.12	-0.16
DRY	-0.26	-0.26	-0.32	0.18	-0.37	-0.05	1.00	0.15	-0.08	0.79**	0.30	-0.06	0.18	-0.27	-0.15	0.30	-0.06	0.36	-0.03	0.01
TAC	-0.02	-0.10	0.14	0.33	0.11	-0.03	0.15	1.00	0.03	-0.02	0.24	0.16	0.05	0.13	0.27	0.06	0.26	-0.05	-0.07	0.50*
TRSC	-0.26	0.18	-0.30	-0.02	0.11	-0.30	-0.07	0.03	1.00	-0.18	-0.19	0.14	-0.02	-0.32	0.04	-0.62**	0.03	0.10	-0.28	-0.05
TRFC	-0.03	-0.36	-0.04	-0.10	-0.17	0.16	0.76**	-0.02	-0.16	1.00	0.20	-0.01	0.24	-0.06	-0.15	0.27	0.08	0.30	0.08	-0.06
WDLR	0.22	-0.04	-0.19	0.22	0.02	0.05	0.30	0.23	-0.18	0.18	1.00	-0.09	0.46*	-0.07	0.44*	0.36	0.46*	-0.19	0.07	0.10
WDL L	-0.02	-0.01	0.20	-0.29	-0.02	-0.23	-0.06	0.16	0.14	-0.01	-0.09	1.00	-0.09	-0.14	-0.12	-0.10	0.00	-0.10	0.05	-0.03
WIVR	-0.02	-0.21	-0.18	0.11	-0.13	-0.23	0.18	0.05	-0.02	0.23	0.45*	-0.09	1.00	0.11	0.65**	0.09	0.62**	-0.35	0.43*	0.05
WIVL	0.09	-0.08	0.52**	-0.09	0.24	0.08	-0.27	0.13	-0.31	-0.05	-0.07	-0.14	0.11	1.00	0.30	0.24	0.27	-0.04	0.13	0.69**
WVR	-0.13	0.07	0.06	0.20	0.04	-0.14	-0.15	0.27	0.04	-0.14	0.43*	-0.12	0.65**	0.30	1.00	-0.12	0.72**	-0.24	0.41*	0.26
WVL	0.24	-0.24	-0.14	0.08	0.01	-0.03	0.30	0.06	-0.60**	0.26	0.35	-0.10	0.09	0.24	-0.12	1.00	-0.08	-0.21	-0.35	0.28
WBR	-0.06	-0.16	0.23	-0.01	-0.17	-0.07	-0.06	0.26	0.03	0.07	0.45*	0.00	0.62**	0.27	0.72**	-0.08	1.00	-0.22	0.35	0.24
WBL	-0.21	0.03	0.14	0.21	0.13	-0.02	0.36	-0.05	0.10	0.28	-0.19	-0.10	-0.35	-0.04	-0.24	-0.21	-0.22	1.00	-0.02	-0.15
WFAR	-0.21	-0.11	0.22	-0.13	-0.19	0.10	-0.03	-0.07	-0.27	0.07	0.07	0.05	0.43*	0.13	0.41*	-0.35	0.35	-0.02	1.00	-0.08
WFAL	-0.09	-0.14	0.12	-0.06	0.21	-0.13	0.01	0.50*	-0.05	-0.06	0.10	-0.03	0.05	0.69**	0.26	0.28	0.24	-0.15	-0.08	1.00

\* Significant at 5% ; \*\* Significant at 1%;

PH Plant Height (cm); NPBP No. of primary branches/plant; D75F Days to 75% flowering; D75M Days to 75% maturity; RL Root length (cm); RD Root Diameter (cm); DRY Dry Root Yield (g/plant); TAC Total Alkaloid Content (%); TRSC Total root starch content (%); TRFC Total root fibre content (%); WDL L Wedelolactone leaves (ng/μl); WDL R Wedelolactone roots (ng/μl); WIV R Withanoside IV roots (ng/μl); WIV L Withanoside IV leaves (ng/μl); WVR Withanoside V roots (ng/μl); WVL Withanoside V leaves (ng/μl); WBR Withanolide B roots (ng/μl); WBL Withanolide B leaves (ng/μl); WFAR Withaferin A roots (ng/μl); WFAL Withaferin A leaves (ng/μl).



Correlation studies play an important role in plant breeding programs used to develop new varieties that use selection that requires the combination of high yield potential with desirable traits. All possible correlations and intercorrelations between morpho-biochemical features are summarized in Table 3. The correlation matrix showed that these traits are more likely to be independent of each other and a higher value of one character may not affect the levels of the other character. A strong positive correlation of dry root yield with total root fiber content at both genotypic and phenotypic levels indicated that the selection practiced to improve these traits directly influences/improves dry root yield.

The path coefficient analysis was performed to assess the direct and indirect extent of the contributions of various traits to the root yield, so that the dry root yield was considered as a dependent trait and the other traits as independent traits. The phenotypic and genotypic coefficients of dry root weight indicating the direct and indirect contribution of various traits are shown in Tables S7-S8 and the corresponding phenotypic pathway diagram for dry root yield with other traits is shown in Fig. 5.

The total root fiber content showed a significantly positive association with the dry root yield in both the genotypic (0.79) and phenotypic path analysis (0.76). Thus, the dry fiber content of the root was the main factor in both pathways analyzes and can

be used as an effective parameter in the direct selection of ginseng genotypes with high root yield.

In addition to other characteristics, the total alkaloid content and WDLR in the genotypic path analysis and WFAL, WBL, WIVR and WDLR in the phenotypic path analysis had a positive direct influence on the dry root yield. Both the genotypic and the phenotypic path analysis of root length, root diameter and WBR had a negative direct influence on the dry root yield. The residual effect of 0.1357 and 0.2482 for the genotypic and phenotypic path analysis showed the influence of other unknown and not considered variables on the root yield.

#### 4. Discussion

In the present study, twenty different traits were used to assess performance of the 25 ginseng genotypes and found that all traits were significant at 1% and 5%. Srivastava et al. (2018a) assessed 53 different genetic stocks of Ashwagandha for 14 quantitative traits using the mean data from 2 years and the ANOVA found that the genotypes for all traits examined were significantly different, similar to the results observed in the present study.

In the present study, for the first time, higher levels of WDL (a potent anti-tumor agent) were reported in both the leaves and

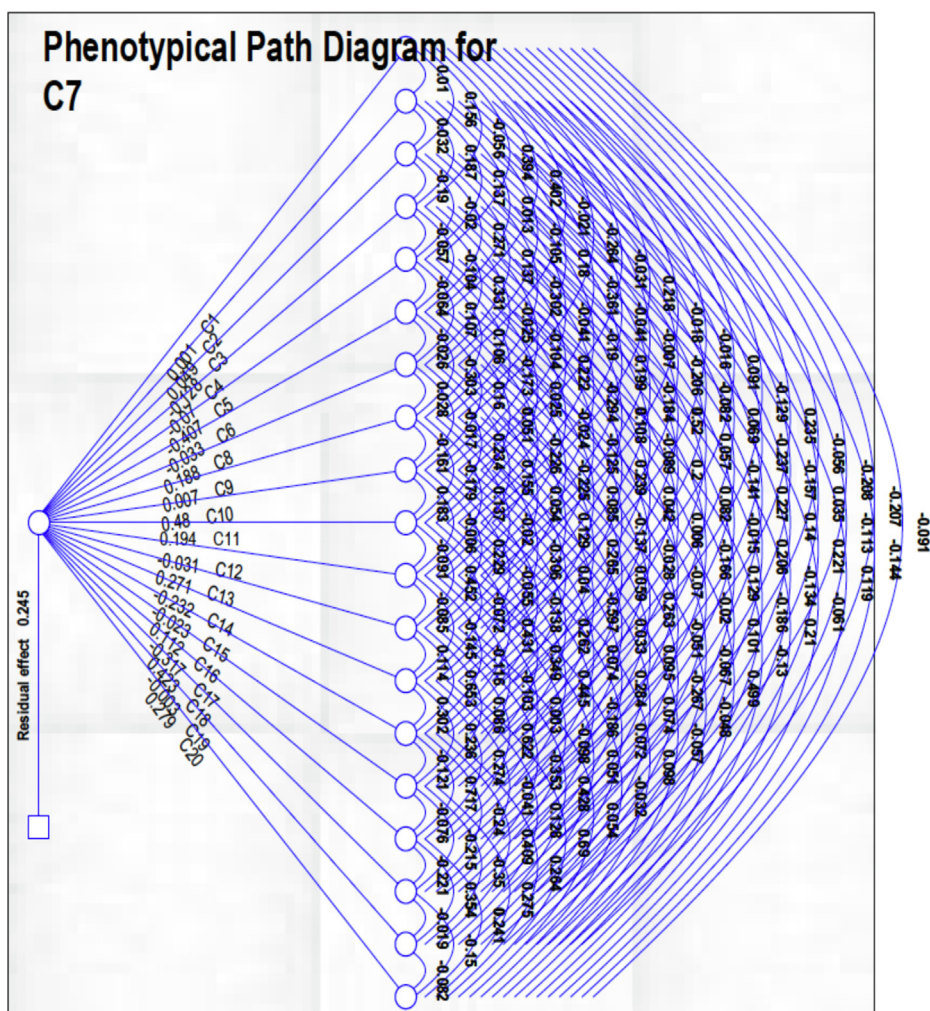


Fig. 5. Path diagram showing the values of direct and indirect contribution of yield and yield-related traits on dry root yield in Indian ginseng. C1 Plant Height (cm); C2No. of primary branches/plant; C3 Days to 75% flowering; C4 Days to 75% maturity; C5 Root length (cm); C6 Root Diameter (cm); C7 Dry Root Yield (g/plant); C8Total Alkaloid Content (%); C9 Total root starch content (%);C10 Total root fiber content (%); C11 WDL leaves(ng/μl); C12 WDL roots(ng/μl); C13 WS-IV roots(ng/μl); C14 WS-IV leaves(ng/μl); C15 WS-V roots (ng/μl); C16 WS-V (ng/μl); C17 WL-B roots(ng/μl); C18 WL-B leaves(ng/μl); C19 WF-A roots(ng/μl); C20 WF-A leaves(ng/μl).

roots of ginseng. Higher withanolide content in leaves could be attributed to the increased expression of triterpenoid biosynthetic genes in leaves than in roots (Srivastava et al., 2018b). In addition, the different withanolide accumulation in different plant parts of Ashwagandha is attributed to the different regulation of withanolide biosynthetic genes, which in turn mediate different above-ground and underground plant interactions/functions in response to extrinsic and intrinsic factors (Dhar et al., 2013; Srivastava et al., 2018b).

Based on morpho-biochemical traits, extensive diversity of 87.5% was found among genotypes, indicating their usefulness in developing selection/breeding strategies. Similar studies were previously carried out by Kumar et al. (2011) in Ashwagandha, Kaur et al. (2016) in green gram and Singh et al. (2020) in garden cress.

The genetic diversity among the 25 genotypes was assessed using RAPD and ISSR molecular markers and these primers can be used to develop a marker system for the rapid and accurate selection of specific high-yielding genotypes. Recently, Hiremath et al. (2021) also reported that the ISSR's molecular marker-based diversity assessment can be used as an efficient tool to demonstrate similarity and phylogenetic relationships between ginseng genotypes collected at different geographic locations. Further, In contrast to previous reports, this study reported a higher polymorphism (85.12%) in ginseng genotypes with both RAPD and ISSR markers. The moderate level of genetic diversity revealed by the Jaccards Similarity Index is attributed to the process of natural selection due to the accumulation of new gene combinations. Molecular breeding using various molecular markers has helped plant breeders test genetic diversity in less time and study the genetic factors that regulate quantitatively inherited traits (Kaur et al., 2016).

GCV, PCV, heritability, genetic advancement, and genetic gain varied widely for all measured traits studied in the present study. The different ginseng accessions were grown under the same environmental conditions, so the differences between the different characteristics can be attributed solely to the genetic makeup of the individual ginseng accessions (Patel and Desai, 2017). A significant contribution of the additive genetic variance, which is generated by the additive gene effect on the expression of traits such as total alkaloid and withanolides, could be used to improve the cultivated plants through selection. These results are in line with previous reports of the ginseng breeding program for root yield and other traits (Joshi et al., 2014; Sangwan et al., 2013; Srivastava et al., 2018a).

## 5. Conclusion

The genetic variability and association were assessed among 25 accessions of Indian ginseng. Phenotypic, genetic, and metabolic traits were studied that used morphological, biochemical, and molecular markers to assess the direct and indirect effects of traits on root weight. ANOVA showed significant differences between 25 different genotypes for the traits examined. Assessment of diversity at the morphomolecular level between ginseng genotypes has been of great importance in developing breeding strategies for both qualitative and quantitative traits. In the present study, high WDL values were reported for the first time with the ginseng additives. The characterization of genotypes through chemoprofiling revealed that UWS-22 and UWS-111 are ideal for the production of alkaloids and withanolides. Path analysis, correlation and variability indicated that dry root yield and total root fiber content determine the diversity among all 20 traits, and these two traits can be used to select superior ginseng genotypes. This information can be used to improve root and specific chemotypes based on the levels of different alkaloids in roots and leaves of ginseng geno-

types, and there is also scope for developing high yielding strains by choosing different parents to cross from the current ones Accessions that can develop high economic value in ginseng.

## CRedit authorship contribution statement

**Surya Chauhan:** Methodology, Writing – original draft. **Trapti Mandliya:** Methodology, Writing – original draft. **Devendra Jain:** Conceptualization, Visualization, Writing – review & editing, Supervision. **Arunabh Joshi:** Validation. **Champa Lal Khatik:** Validation, Formal analysis. **Abhijeet Singh:** Conceptualization, Visualization, Supervision. **Sudhir K. Upadhyay:** Formal analysis, Writing – review & editing. **Rohit Jain:** Methodology, Writing – original draft.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

Financial assistance from Manipal University Jaipur in the form of Seed Grant EF/2017/QE04-03 is gratefully acknowledged.

## Source of funding

The author covers the full cost of this work.

## Data availability

The data that support the findings of this study are available from the author upon a reasonable request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2022.01.030>.

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