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Interpopulation Variation in *Picrorhiza kurrooa* Royle ex Benth-Step Towards Identifying Genetic Variability and Elite Strains for Crop Improvement Study*

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Abstract: This communication addresses variability at morphological, biochemical and active ingredients level among wild populations of *Picrorhiza kurrooa*, an important medicinal herb of high altitude region of Himalaya. Variations were observed for microhabitats as well as for two wild strains viz., narrow leaf and broad leaf. Observations suggest variability in vegetative growth while fruits and seeds do not impart significant variation among different populations, microhabitats and strains. Diverse relationship between some morphological characters was noticed viz., leaf characters showed directly proportional relationship with fruit and seed characters. Results reveals almost same pattern of seed peroxidase isoenzyme of both broad leaves and narrow leaves strains even though some populations have different intensity and color bands. Number, intensity and position of esterase isoenzyme band of broad leaves strain do not manifest any specific difference which may suggest genetic polymorphism. Even though, observations are of primary level for biochemical analysis, different intensity bands may advocate genetic variability and adaptive feature of the populations in varying geographical regions. There was considerable variation in active ingredients of both strains and among the populations. Maximum numbers of active components (18) were detected in narrow leaves strain and both broad leaves and narrow leaves strains of *Picrorhiza kurrooa* from VF population were characterized by the maximum picrotin and picrotoxin contents. Correlation matrix suggests significance of certain morphological characters i.e. plant height, leaf density and area for better alkaloid contents while, flowers adversely affects alkaloid contents. It is also suggested from the findings that moisture regime of raw drug (stolon in *P. kurrooa*) also determine the quality and can be taken into account for appropriate drying techniques. Overall results indicate superiority of broad leaves strain over narrow leaves strain regarding the morphological features and active contents.

Key words: Active ingredients, isoenzyme, microhabitats, stolon, variation

INTRODUCTION

In extreme climatic and ecological conditions, plants of alpine zone possess attractive appearance, interesting mode of perennation and special morphological, physiological and adaptational features. Region harbors several medicinal plants and more importantly, the plants of this zone synthesize secondary metabolites of medicinal importance and therefore, offer greater possibilities of having novel bimolecules and even larger quantity of active components (Nautiyal *et al*., 2005). Plants adopt several adaptive strategies to cope with alpine environmental conditions and it is considered that mass

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flowering (Kuniyal *et al*., 2003) and fruit characteristics are associated with different environmental conditions and genotype of a species (Singh *et al*., 1999). Furthermore, seed production of a species may indicate a compromise between ecological and physiological parameters (Siemens and Johnson, 1995; Kuniyal *et al*., 2003). As a result, variation in morphology, physiological and biochemical characters appear in many alpine plants. Electrophoretic results have revealed large amounts of genetic polymorphism in natural populations (Nevo, 2001) morphological and biochemical variations along with considerable polymorphism in seed polypeptides and esterase isoenzyme characterization in high altitude plants have been reported (Kuniyal *et al*., 2001, 2002). Variations among the populations of some high altitude species have been reported (Kuniyal *et al*., 2003; Nautiyal *et al*., 2003; Vashistha *et al*., 2006) and therefore, efforts are desirable to explore these variations at morphological, biochemical and secondary metabolites level to explore the level of genetic diversity and more importantly, to evaluate these natural populations for the selection of superior and elite germplasm for domestication and cultivation particularly of important medicinal herbs including *Picrorhiza kurrooa*.

Picrorhiza kurrooa Royle ex Benth (Family Scrophulariaceae) a perennial herb with an elongate, stout and creeping stolon is found in the subalpine - alpine Himalaya from Kashmir to Sikkim at an altitudes of 2700-4500 m and commonly known as Kutki or Kadwi. It furnishes the drug *Picrorhiza* obtained as dried stolons. Information on taxonomy, distribution, uses, cultivation practices and active ingredient analysis of the species is available (Naithani, 1985; Kaul and Kaul, 1996; Nautiyal *et al*., 2001; Nautiyal and Nautiyal, 2004). It is considered to be a valuable bitter tonic almost as efficacious as gentian. It is antiperiodic, cholagoque, stomachic, laxative in small doses and cathartic in large doses (Hussain, 1984; Ram, 2001). National Medicinal Plants Board (NMPB) estimated annual demand of 220.3 tonnes in 2001-02 and 317 tonnes during 2004-05 in India with annual growth rate of 12.9% in species demand. A fairly large quantity of the drug is collected from various places in the North-west and North-east Himalaya. This has led to the unscientific extraction of plant which adversely affects the availability in wild therefore, declared as endangered species (CAMP, 2003). CITES included this in its Annexure II and complete ban is imposed by Govt. of India for harvesting from wild. Therefore, attempts are required at earliest to conserve various wild strains and in chorus to cultivate it for the medicinal uses. This needs to evaluate the wild populations for the identification of elite germplasm for crop improvement so that all possible strains could be conserved. This study communicates to identification of all possible microhabitats, to observe morphological variations among different microhabitats and locations, to evaluate biochemical variations, to qualitative and quantitative evaluation of all populations for the glycoside contents and to find out relationship between morphological, biochemical and active ingredients of *Picrorhiza kurrooa*.

MATERIALS AND METHODS

Study Area and Microhabitats of *P. kurrooa* in Wild

Entire subalpine-alpine region of Garhwal Himalaya (77°33'5" to 80°6'E longitudes and 29°31'9" to 31°26'5" N latitudes) known for the occurrence of the species was surveyed during 2005-06 and five locations were selected further for the observations. These sites were Tungnath (TN, 2800-3600 m msl) and Kilpur (KIL, 2800-3400 m msl) in Rudraprayag district, The Valley of Flowers (VF, 2700-3600 m msl) and Kuwari Pass (KP, 2800-3800 m msl) in Chamoli district and PanwaliKantha (PK, 2700-3400 m mal) in Tehri district of Garhwal, Uttrakhand, India. In each location, three microhabitats of *P. kurrooa* were identified based mainly on topography and vegetation cover i.e., (i) Rock Surfaces (RS) having steep slopes, thin soil cover and grass dominated vegetation type, (ii) Under Canopy of Scrub (UCS) mainly near timberline or under scrubs of *Quercus semecarpifolia* and *Rhododendron companulatum* with moderate slopes and (iii) Near Springs (NS) microhabitats with

Narrow Leaf (NL) strain

Broad Leaf (BL) strain

Fig. 1: Two strains of *P. kurrooa* in wild population

steep slope, shallow soil depth across the water channels in alpine areas between 3000-3600 m altitude. Two species of *Picrorhiza* viz., *P. kurrooa* and *P. scrophulariiflora* (syn *Neopicrorhiza scrophulariiflora*) are reported in active trade in India and Nepal even though both are reported to be morphologically similar (Olsen, 2001). Nautiyal *et al.* (2001) and Nautiyal and Nautiyal (2004) also identified two strains namely broad leaf (BL) and narrow leaf (NL) strains of *P. kurrooa* (Fig. 1) and therefore, all observations were carried out separately for these two strains.

Morphological Variations among Different Natural Populations

For morphological variations, all populations were surveyed within 10-15 days during the month of July at the time of flowering so that plants identified for observations could be of similar age. Ten plants from each site were marked and different morphological characters viz.-plant height (PH), No. of Leaves (NL), Leaf Length (LL) and width (LW), No. of Flowers (NF), Capsule and seeds per plant and moisture content (MC%) and dry weight (DW) of stolons were observed following Airi *et al.* (2000) and Nautiyal *et al.* (2003). Data were further analyzed for variation using student F-test and relationship among morphological and alkaloid contents were derived using correlation matrix with the help of SPSS package.

Biochemical Studies

Seeds were harvested for Isoenzyme and protein content analysis from wild populations after maturation during late August-September. Seeds weighting 500 mg were homogenized in 5 mL extraction buffer of 0.05 M Tris-HCl buffer, pH 7.2 containing 0.5 M sucrose, 25 mM ascorbic acid

and 0.2% β-mercaptoethanol. The extracts were centrifuged in a centrifuge at 10000 Xg for 20 min. The supernatant was used for the soluble proteins and also for electrophoresis of Isoenzyme. The method described by Bradford (1976) was used for quantitative estimation of proteins.

For all the populations same amount of protein (100 μg) loaded on to 10% polyacrylamide slab gels according to the methods described earlier (Bhadula and Sawhney, 1987). Esterase bands were visualized by the method of Bhadula and Sawhney (1987). Similarly, peroxidase isoenzymes were separated on 10% polyacrylamide slab gels as described by Davis (1964) and detected on by the method of Welter (1982).

Qualitative and Quantitative Assessment of Glycosides

Powdered plant material (dry stolons) was extracted using 70% ethanol in a soxhlet apparatus for 1 h on water bath maintained at a temperature of about 60°C. The extract thus obtained was filtered and dried in vacuum pressure and stored in desiccators until analysis. The dry crude extract (0.1 mg) was dissolved in 10 mL of water: Methanol: isopropanol: acetonitrile in the ration of 60:30:5:5 and from this stock solution 10, 20, 40, 80 and 100 ppm solutions were prepared. Picrotin and picrotoxin (Sigma chemical) were used for establishing the calibration curve. The standard HPLC system consisted 125 Beckman System Gold was used for analysis. A mixture of water: methanol: isopropanol: acetonitrile (60:30:5:5) as a solvent for an elution at a flow rate of 1 mL min^{-1} was used. Twenty microliter of sample solution was injected and glycosides were detected at λ_{max} 220 mm. Components were identified by simultaneous run of standards with its retention time.

RESULTS AND DISCUSSION

Morphological Variations among Different Natural Populations

It implies that RS microhabitats with more exposure to sun light as compared to USC and NS microhabitats, endows with maximum PH, NL, NF, NCPP, NSPC and NS of broad leaves populations (Table 1). However, maximum leaf area was recorded in USC microhabitat of VF population reimbursed for optimal photosynthetic activity under the canopy. Populations responded differently for morphological characters as seed pod and seeds recorded maximum in TN population, flowers in KP and stolon dry weight in PK and KP populations. ANOVA suggested non significant variation in PH, NCPP, NSPC and NS across the populations in all three microhabitats while leaf characteristics (NL, LL, LW), NF and stolon characters (MC and DW%) showed significant variation. Likewise, RS provides maximum PH, NL, NF, NSPC and NS in narrow leaves populations while LL and LW was recorded maximum in USC. Variation among different populations and microhabitats for PH and stolon characters was detected significant on the basis of ANOVA while flower, capsule and seed number had different responses although mostly with non significant variation. In general, results indicated variation in vegetative growth and stolon while flowers and seeds characters did not show significant variations in both strains of *P. kurrooa*.

Correlation matrix ($p = 0.05$) further suggested significant positive relationship between PH vs. NL, LL and LW; NL vs. LL and LW; NCPP vs NSPC and NS. However, PH vs NF; LL and LW vs NF; LW vs NCPP, NSPC and NS and NL vs NS had strong negative correlation in broad leaves strain (Table 3). In contrast, positive relationship was observed only between PH vs LW; LL vs NSPC and NS and NCPP vs NS in narrow leaves strain. Only LL vs NL; NL vs NSPC and LW vs NCPP had significant negative correlation in this strain of *P. kurrooa* (Table 3). PH further imparts positive relationship significantly with moisture content of both broad leaves ($R^2 = 0.77$) and narrow leaves ($R^2 = 0.85$) strains. However, negative relationship was detected between PH and stolon DW even though it was not significant. Among the populations and microhabitats, relationship between PH and

Table 1: Morphological variations in different populations of *Picrorhiza kurrooa*

Microhabitats	Populations	PH (cm)	NL	LL (cm)	LW (cm)	NF	NCPP	NSPC	NS	MC (%)	DW (g plant^{-1})
BL strain											
RS	TN	13.8±1.4	7.6±1.6	6.7±0.5	2.1±0.2	13.4±3.4	10	74	740	67.7±0.1	1.2±0.1
	KIL	14.2±1.3	11.6±1.6	5.5±0.6	2.0±0.1	15.2±4.1	8	58	464	67.7±0.6	1.2±0.8
	VF	14.1±0.8	12.4±0.5	5.8±0.5	2.6±0.1	14.0±1.4	9	58	522	67.5±0.8	1.2±0.0
	KP	11.9±1.8	7.2±1.9	5.4±0.5	2.1±0.3	21.2±3.3	9	60	540	66.9±0.8	1.3±0.9
	PK	13.9±1.0	10.0±1.4	5.9±0.5	2.0±0.1	17.2±3.3	9	60	540	67.7±1.3	1.4±0.8
	F-value	2.76^{ns}	0.31*	28.6*	21.6*	0.16*	6.6^{ns}	1.25^{ns}	0.0^{ns}	11.6*	181.0*
		p>0.1	p>0.1	p<0.01	p<0.01	p<0.1	p<0.01	p<0.5	p<0	p<0.1	p<0.1
NS	TN	13.1±2.2	7.6±1.5	5.8±0.3	2.1±0.2	13.8±3.0	9	74	656	67.7±0.1	1.2±0.1
	KIL	13.6±1.3	8.8±1.7	6.8±0.7	2.2±0.2	15.6±3.2	8	58	464	67.7±0.6	1.2±0.0
	VF	13.0±1.3	11.2±1.1	7.0±0.5	2.3±0.1	11.6±0.8	8	58	464	67.5±0.8	1.3±0.1
	KP	11.5±1.5	6.6±1.3	4.9±0.5	2.0±0.3	18.6±4.1	9	60	540	66.9±0.8	1.2±0.1
	PK	13.5±1.1	10.4±1.6	6.5±0.4	2.1±0.2	17.6±2.6	9	60	540	67.7±1.3	1.3±0.1
	F-value	3.36^{ns}	0.68*	0.68*	3.32^{ns}	154*	8.33^{ns}	0.05^{ns}	0.0^{ns}	21.83*	833*
		p>0.1	p<0.5	p<0.5	p<0.5	p<0.0	p<0.1	p<0.0	p<0.0	p<0.0	p<0.0
USC	TN	13.3±1.5	6.6±0.8	5.8±0.4	2.0±0.1	16.4±2.5	10	64	640	67.7±0.6	1.2±0.8
	KIL	12.5±2.4	9.6±2.4	5.6±1.7	2.4±0.8	18.0±5.0	9	61	549	67.5±0.8	1.2±0.0
	VF	13.2±0.6	11.6±1.6	10.4±0.8	3.2±0.3	13.4±0.8	8	58	464	66.9±0.8	1.3±0.9
	KP	10.3±2.2	6.8±1.1	4.1±0.4	1.6±0.2	15.2±5.0	9	66	594	67.7±1.3	1.4±0.8
	PK	12.9±2.4	9.6±2.4	5.6±1.7	2.4±0.8	18.0±5.1	9	67	603	67.7±0.1	1.2±0.1
	F-value	1.5^{ns}	0.55*	0.44*	6.6*	0.65*	5.0^{ns}	0.18^{ns}	0.0^{ns}	21.83*	312*
	p<0.05	p<0.5	p<0.5	p<0.5	p<0.05	p<0.5	p<0.1	p<0.1	p<0.0	p<0.0	p<0.0
NL strain											
RS	TN	9.5± 0.7	6.4±1.1	6.2±1.2	1.0±0.1	11.6±1.6	7	60	420	70.1±0.0	0.74±0.41
	KIL	10.1±1.1	12.0±2.4	6.7±0.6	1.5±0.1	14.2±3.1	9	64	576	70.1±0.8	0.74±0.00
	VF	11.6±1.4	8.4±1.8	6.4±0.3	1.3±0.1	10.8±1.1	8	60	480	70.2±0.0	0.77±0.12
	KP	11.0±0.7	8.6±2.6	5.3±1.3	1.3±0.1	13.4±2.0	7	65	455	70.4±0.4	0.74±0.03
	PK	10.0±0.9	7.2±1.1	6.5±0.9	1.7±0.2	14.8±2.6	6	67	402	70.1±0.0	0.73±0.02
	F-value	3.5^{ns}	0.55*	8.41*	36.7*	0.85*	1.92^{ns}	0.25*	0.0^{ns}	147*	1086*
		p>0.1	p>0.5	p>0.1	p<0.1	p>0.5	p<0.5	p>0.1	p=0.0	p = 0.0	p = 0.0
NS	TN	9.7±0.9	6.6±0.8	7.2±4.1	1.7±0.2	10.8±1.1	9	50	450	70.2±0.0	0.77±0.12
	KIL	9.9±1.4	7.0±1.0	6.6±0.7	1.4±0.1	13.8±3.0	9	61	549	70.4±0.4	0.74±0.03
	VF	10.5±1.4	9.2±2.2	5.5±0.9	1.6±0.2	12.0±1.4	8	60	480	70.1±0.0	0.73±0.02
	KP	9.3±1.7	9.8±3.7	3.7±1.4	1.6±0.4	12.0±2.5	9	49	441	70.1±0.8	0.74±0.00
	PK	9.8±0.7	8.0±2.4	6.8±0.5	1.5±0.1	13.2±2.2	8	65	520	70.2±0.0	0.77±0.12
	F-value	13.29*	1.32^{ns}	1.25*	192*	1.82^{ns}	8.33*	0.04^{ns}	0.0^{ns}	166*	7142*
		p>0.01	p<0.5	p<0.5	p = 0.0	p<0.5	p<0.05	p = 0.0	p=0.0	p = 0.0	p = 0.0
USC	TN	10.0±0.9	5.6±0.5	5.0±0.6	1.5±0.3	11.8±1.1	9	60	540	70.4±0.0	0.74±0.03
	KIL	10.0±1.2	10.0±2.0	5.4±1.5	1.4±0.2	13.4±2.9	8	41	328	70.2±0.0	0.77±0.12
	VF	10.8±1.2	11.6±0.8	7.3±0.8	1.8±0.2	10.2±0.4	7	45	315	70.4±0.4	0.74±0.03
	KP	9.3±1.7	12.2±5.6	4.0±1.3	1.5±0.3	12.6±3.7	6	48	288	70.1±0.0	0.73±0.02
	PK	9.4±1.3	7.6±1.6	6.3±0.7	1.4±0.1	12.2±2.2	9	45	405	70.2±0.0	0.77±0.12
	F-value	6.94*	0.32*	1.57^{ns}	92.5*	1.77^{ns}	1.47^{ns}	0.04^{ns}	0.0^{ns}	138*	7142*
	p<0.05	p<0.05	p>0.1	p<0.5	p = 0	p<0.5	p<0.5	p = 0	p = 0	p = 0	p = 0

PH: Plant Height, NL: No. of Leaves, LL: Leaf Length, LW: Leaf Width, NF: No. of Flowers, NCPP: No. of Capsule per Plant, NSPC: No. of Seed per Capsule, NS: No. of Seeds, MC: Stolon Moisture Content, DW: Stolon Dry Weight, ±SD, *Significant, ns-Non significant

economic yield (DW of stolon) was found significant (R^2= 0.71; p= 0.05) only in USC microhabitat. Plants grown under the shade of timberline trees (USC microhabitat) had better growth with large leaves and root system and vigorous stoloniferous growth of stolons probably due to humus of dead leaves and protection form direct sun light as observed by Airi *et al*. (2000) higher moisture content (Nautiyal *et al*., 1997) and rich in organic carbon and nitrogen (Ram and Singh, 1994). Similar observation was noted earlier for high altitude medicinal plant *Podophyllum hexandrum* by Airi *et al*. (1997). Results summarizes that though morphological characters has diverse relationship, in some cases viz., between leaf characters and fruits capsule seed in particular relationship was directly proportional.

In general, broad leaves strain had maximum plant height, leaf area, number of flowers and seed productivity in all populations as well as in microhabitats as compared to narrow leaves strain of *P. kurrooa*. Purohit *et al*. (1999) reported considerable variation in leaf morphology in different populations of *Podophyllum hexandrum* in Garhwal Himalaya. Variations in the length and width of leaves in the plants are supposed to be sensitive to the varying environmental conditions (Lynn and Waldren, 2001). Similarly, production of more flowers has been considered an adaptive feature in the high altitude plants subjected to the variety of stress conditions (Kuniyal *et al*., 2003). It is argued that variation in the plant morphology appears throughout the ontogeny. However, variations in some species may peak at the middle of growth period. In addition, available soil nutrient level also plays important role in determining morphological variations in plants (Pigliucci *et al*., 1997; Kuniyal *et al*., 2002), climatic factors (Krishnan *et al*., 2000) and can be considered as indicator to alter assimilate investment pattern (Korner *et al*., 1989).

Native Electrophoresis

Biochemical variations suggested polymorphic nature of the species and it is argued that they can be used as an indicator to the impact of microclimatic conditions on plant life (Kuniyal *et al*., 2002) and therefore, reflects an adaptive feature of the species (Bhadula *et al*., 1981). Esterase, peroxidase, acid phosphates (Barone *et al*.,1996) and isoenzyme (Bousquet *et al*., 1986; Godt and Hamrick, 1995) can be used for the identification of germplasm and genetic diversity. Isoenzymes are frequently used to characterize germplasm and determination of genetic diversity (Brown, 1978; Souza and Sorrells, 1989).

It reveals that seed peroxidase isoenzyme of both broad leaves and narrow leaves strains have almost same pattern. However, the intensity and position of the band was different in PK population of narrow leaves strains (Fig. 2A). Likewise, KIL and PK populations of broad leaves strain have higher colour intensity of bands in contrast to the rest of the populations (Fig. 2B).

Number, intensity and position of esterase isoenzyme band of broad leaves strains do not manifest any specific difference (Fig. 3B) may suggest genetic polymorphism. However, barring TN and KIL populations of broad leaves, other populations (viz., VF, KP and PK) have esterase bands of different intensity (Fig. 3A). Even though our observations are of primary level for biochemical analysis, different intensity bands may advocate genetic variability and adaptive feature of the populations in varying geographical regions as suggested earlier by Bahuguna *et al*. (2003) in *Aconitum* species of alpine. A similar observation has been made by Vashistha *et al*. (2007) for two *Angelica* species. Nevo (2001) suggested that genetic diversity is higher in species living in broader environmental spectra or species with a patchy population structure and limited migration.

Plants contain several isoenzymes whose pattern of expression is tissue specific and developmentally regulated or responsive to environmental stimuli (Lagrimini and Rothstein, 1987). Peroxidase, which are widely distributed in plants play major role in the biosynthesis of cell wall polymers (Liyama *et al*., 1993) are implicated in wound healing processes (Lagrimini and Rothstein, 1987) and may be involved in auxin catabolism or defense against pathogen attack (Grisebach, 1981). Peroxidase, which is generally composed of a number of isoenzymes, is capable of catalyzing several types of metabolic activities and also involved in synthesis of ethylene.

Variations in Glycosides Contents among the Natural Populations

Table 2 shows quantitative and quantitative data of active ingredients. It appears that, both strains (BL and NL) of *Picrorhiza kurrooa* from VF population were characterized by the maximum picrotin and picrotoxin contents. Picrotoxin content was 2.20 mg g^{-1} for broad leaves as well as narrow leaves strains in this population while picrotin content was almost 2.4 time more in broad leaves strain as

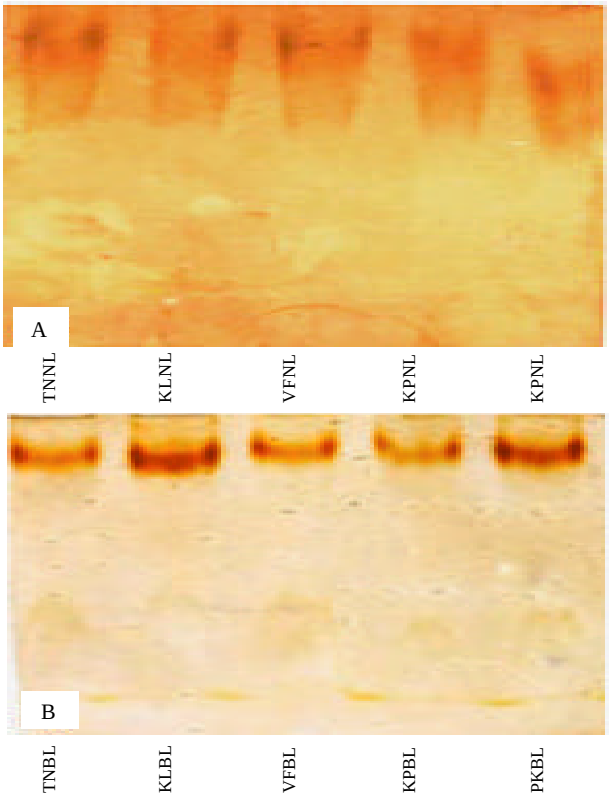

Fig. 2: Peroxidase isoenzyme pattern in the seeds of *P. kurrooa* (NL (A) and BL (B) collected from different populations, TN: Tungnath, KIL: Kilpur, VF: The Valley of Flowers, KP: Kuwaripass, PK: Panwali Kantha

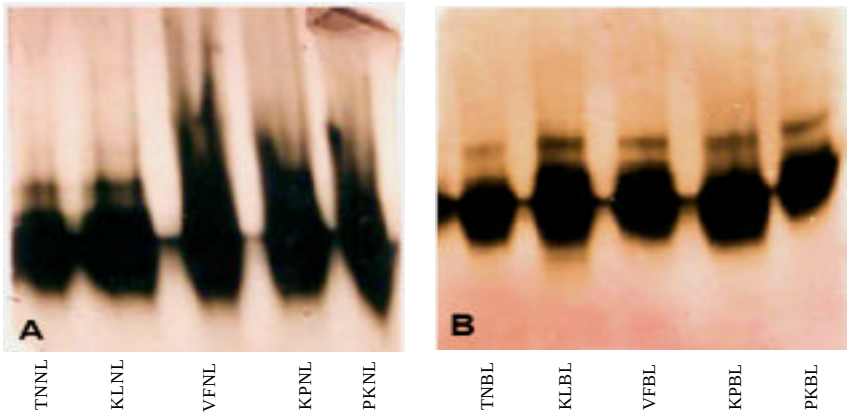

Fig. 3: Esterase isoenzyme pattern in the seeds of *P. kurrooa* (NL (A) and BL (B) collected from different populations, TN: Tungnath, KIL: Kilpur, VF: The Valley of Flowers, KP: Kuwaripass, PK: Panwali Kantha

Table 2: Variation in active ingredients in different populations of *Picrorhiza kurrooa*

Active ingredients	Populations				
	TN	VF	KIL	PK	KP
BL					
No. of components	15.00	17.00	14.00	14.00	15.00
Picrotin (mg g^{-1})	2.25	3.85	2.55	2.35	1.00
Picrotoxin (mg g^{-1})	1.30	2.20	2.00	1.30	0.15
NL					
No. of components	16.00	18.00	16.00	18.00	14.00
Picrotin (mg g^{-1})	1.10	1.60	1.00	1.00	0.80
Picrotoxin (mg g^{-1})	1.25	2.20	0.40	1.50	0.20

Table 3: Correlation matrix between morphology and alkaloid contents in wild populations of BL and NL strains of *P. kurrooa*

	Morphological characters*										Glycosides characters		
	PH	NL	LL	LW	NF	NCPP	NSPC	NS	MC	DW	P	PT	NC
PH	1	**0.47**	**0.28**	**0.63**	**- 0.57**	**- 0.17**	**- 0.39**	**-0.22**	**0.85**	**- 0.06**	**0.90**	**0.63**	**0.47**
NL	0.64	1.00	**- 0.52**	**0.19**	**0.09**	**- 0.26**	**- 0.85**	**- 0.47**	**0.39**	**- 0.67**	**0.04**	**- 0.35**	**- 0.38**
LL	0.77	0.81	1.00	**0.28**	**- 0.06**	**0.48**	**0.64**	**0.66**	**0.26**	**0.86**	**0.57**	**0.67**	**0.88**
LW	0.56	0.89	0.93	1.00	**- 0.17**	**- 0.62**	**0.17**	**- 0.49**	**0.15**	**0.36**	**0.57**	**0.68**	**0.68**
NF	-0.59	-0.47	-0.88	-0.78	1.00	**0.21**	**0.24**	**0.24**	**- 0.62**	**0.21**	**- 0.69**	**- 0.63**	**- 0.17**
NCPP	-0.14	-0.77	-0.38	-0.63	0.16	1.00	**0.10**	**0.95**	**0.25**	**0.21**	**- 0.00**	**- 0.17**	**0.03**
NSPC	0.03	-0.70	-0.25	-0.53	-0.01	0.96	1.00	**0.37**	**- 0.53**	**0.92**	**- 0.06**	**0.39**	**0.62**
NS	0.06	-0.72	-0.35	-0.64	0.08	0.86	0.94	1.00	**0.12**	**0.47**	**0.03**	**- 0.01**	**0.24**
MC	0.76	0.18	0.18	-0.06	0.00	0.18	0.29	0.44	1.00	**- 0.24**	**0.82**	**0.42**	**0.23**
DW	-0.33	0.13	-0.06	0.08	0.32	0.00	-0.24	-0.46	-0.37	1.00	**0.23**	**0.56**	**0.85**
P	0.77	0.87	0.98	0.95	-0.83	-0.51	-0.37	-0.44	0.19	-0.09	1.00	**0.86**	**0.69**
PT	0.86	0.83	0.87	0.82	-0.73	-0.56	-0.36	-0.31	0.41	-0.38	0.92	1.00	**0.87**
NC	-0.01	0.33	0.61	0.70	-0.73	-0.24	-0.21	-0.44	-0.63	0.21	0.56	0.27	1.00

*As per Table 1, P: Picrotin, PT: Picrotoxin, NC: No. of Components; Bold values of the table represents the value of correlation coefficient of NL and non bold values for BL strains; p = 0.05

compared to narrow leaves strain. In general, there was considerable variation in active ingredients of both strains and in populations. However, maximum numbers of active components (18) were detected in narrow leaves strains from two populations, VF and PK. Picrotin and picrotoxin contents showed strong positive correlation as seen in Table 3 with certain morphological characters viz., PH, NL, LL and LW although these contents had significant negative correlation with flowers and seed characters (NF,NCPP) on the basis of correlation matrix (P=0.05) in broad leaves strain. Further, P had significant positive relationship with PT (R^2 = 0.92) and NC (R^2 = 0.56) and moisture of stolon adversely affected alkaloid contents as there was significant negative correlation (R^2 = -0.63) between MC and NC. Narrow leaves strain exhibited similar pattern with strong proportional relationship of P, PT and NC with PH, LL and LW; P with MC of stolon ((R^2 = 0.81) and PT (r^2 = 0.56) and NC (R^2 = 0.84) with DW of stolon at P= 0.05. P,PT and NC had negative relationship with NF. However, NC was in proportion to NC with R^2 = 0.61 at P=0.05 level in NL strain. Study revealed that broad leaves strain had better glycoside contents than narrow leaves strain and VF population had best quality of germplasm and this elite germplasm can be used for domestication and for multiplication of germplasm for commercial cultivation. Correlation matrix suggests significance of plant height, leaf density and area for yielding better alkaloid contents. Likewise, presence of flower adversely affects alkaloid contents. It is also suggested from the findings that moisture regime of raw drug (stolon in *P. kurrooa*) also determine the quality and can be taken into account for appropriate drying techniques.

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