Progress Report

Germplasm evaluation of *Lathyrus aphaca* L., a minor crop species of local importance

Lathyrus aphaca L. is an annual herb with a short life cycle of 4-5 months. Plants bear tap roots laden with nodules. The leaves are modified into tendrils which curl/coil, provide support and help in climbing. Whereas the stipules modified into leaves are green and support photosynthesis. Arranged in opposite manner, each stipule has an acute apex and entire margin. Plants of ten populations belonging to five different districts of Jammu and Kashmir and one of Punjab were chosen for present investigation. These ten populations belong to Ratwana and Nichla (Samba district), Krishna Colony and Rajbagh (Kathua district), Aghar Jitto (Reasi district), G.D.C Rajouri (Rajouri district), Campus of University of Jammu and Jhiri (Jammu district) and Bani Lodhi and Sunderchak (Pathankot district) of Punjab (Table 1).

Table 1 Sources of collection and details of their ecological correlates

S. No.	Districts	Site of collection	Population designated as	Ecological correlates	Altitude masl	Habitat
1.	Jammu	a)Campus of University of Jammu	JU	N32° 72.66′ E74° 85.22′	400	Wasteland
		b)Jhiri	JH	N 32 49.509 E74 43.5608	327	Along sides of wheat field
2.	Kathua	a)Krishna Colony	KA	N32° 23.149′ E75° 31.076′	332	Along sides of wheat fields
		b)Rajbagh	RJ	N 32^0 36728 E 75^0 53748	393	Along sides of wheat fields
3.	Samba	a) Ratwana	RW	N32° 33.650′ E75° 15.791′	455	Within fields of wheat
		b) Nichla	NI	N32° 32.754′ N75° 31.076′	414	Open field
4.	Reasi	Aghar jitto	RE	N32 550.41 E7456 52.87	689	Wasteland
5.	Rajouri	GDC Rajouri	RA	N33 39.2133 E74 32.1337	955	Botanical Garden
6.	Pathankot	a)Bani Lodhi	BL	N 32^0 2838′ E 75^0 5379′	335	Within sides of wheat fields
		b)Sunderchak	SD	N 32^0 27.07′ E 75^0 5354′	330	Within sides of wheat fields

a) RO

b) RW

c) KA

d) NI

Fig.1a-d Plants of some populations; RO(Rajouri), RW(Ratwana), KA (Kathua) and NI (Nichla)

Fig.2An individual plant of *Lathyrus aphaca*

Fig.3 Mature flowers of *L.aphaca* of varying sizes

Fig.4 Fruit with healthy seeds

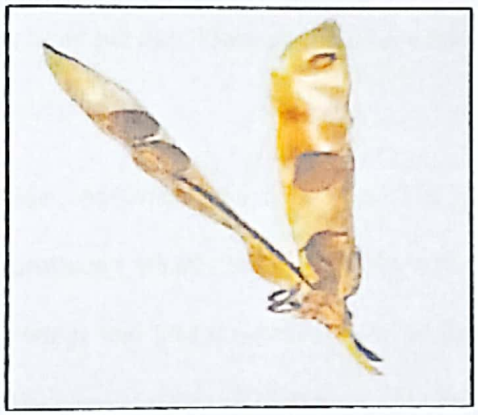

Fig.5 Fruit with aborted seeds

Morphological studies:

The plants of these populations were worked out for their morphological and cytological traits.

Of the 10 populations, plants of RW are more than 100 cm in height where as those of JU, KA,

SD, BL, JH, RA are more or less equal in height and of NI the smallest ($\overline{Y}$=13.3 cm). The number of branches also varied among populations. RW had 38.6 branches which is the highest across populations. The average number of stipules varied from 20.5 to 840.62. The number of flowers per plant differed widely with an average of about 6 in NI and 106 in RW. Plants of JU, KA, RA, RE, BL, SD, RJ, JH are half the size of RW, while those of NI are tiny (see Figs 1a-d). They account for one-fourth of RW plants in height and other morphological attributes.

Floral morphology and phenology

The plants have a very short flowering period of 15-20 days. Initiated by the end of February flowering reaches its peak in March. Flowers are solitary axillary, small, pale yellow, bisexual, zygomorphic and hypogynous. Flowering is asynchronous i.e., all the flowers do not bloom at the same time (Fig.2). Only 2-3 flowers bloom per plant per day. Flowers at the base mature first followed by those at the apex and vary in overall size (Fig.3).

An individual flower is pentamerous, light yellow, odourless and nectarless (Fig.3). Calyx consists of five green sepals fused at the base. Corolla is typically papilionaceous with 5 petals differentiated into outermost standard, two inner wings and two innermost fused to form keel. Sexual organs are concealed within this keel. Androecium consists of 10 stamens in diadelphous condition. Nine fused by their filaments form a 7.1 to 10.7 mm long staminal tube while the tenth is free and smaller as compared to the fused stamens. Gynoecium is monocarpellary. The 7.8 to 12.7 mm long pistil exceeds the lengths of both the fused and free stamens. Pistil is composed of a small stigma, straight dorsally compressed style and an ovary.

Reproductive biology:

Floral events including anthesis, anther dehiscence and stigma receptivity were also studied. Anthesis begins at 0800 hrs in the morning and is marked by an expansion of the standard. The anthers dehisce before the stigma gets receptive making the flower protandrous. Different pollination treatments were also conducted like manual self pollination, manual cross pollination, emasculation and bagging etc. The results revealed the plants to be self as well as cross pollinated. Fruits bear healthy and aborted seeds (Figs 4,5).

Cytological studies

A total of 1,281 pmcs were encounterd for meiotic studies and all the pmcs exhibited normal meiosis in the male track. These pmcs invariably had 7IIs at diplotene, diakinesis and metaphase-I which segregated equally and normally at anaphases-I and II (Fig.6). All the good preparations were scored and photographed. Interestingly, somatic complements of all the plants also showed normal chromosome count of 2n=14(Figs 7a,b). A total of 200 tips were squashed for mitosis and all the cells showed uniform count of 14 chromosomes. Majority of the chromosomes were submetacentric whereas few subtelocentric chromosomes were also observed. Inspite of having same chromosome number, minute karyotypic differences were observed in TCL as well as MCL(Fig 7b). Pollen mitoses also revealed the presence of seven chromosomes at metaphase.

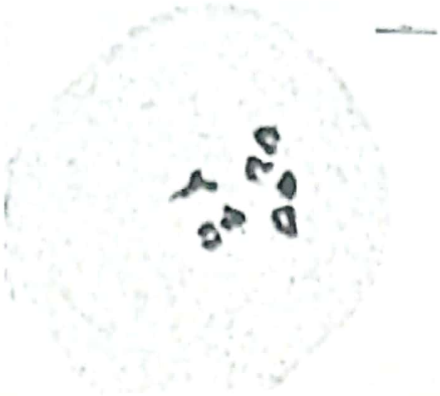

Fig.6 A pmc at metaphase-I showing 7IIs chromosomes

Fig.7 (a) A Root tip cell at metaphase showing 2n=14

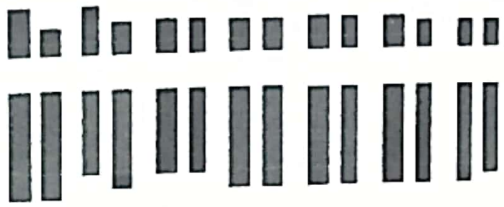

Fig.7 (b) Idiogram with 2n=14

Biochemical studies:

The seeds of *Lathyrus aphaca* were also subjected to a preliminary biochemical analysis, which revealed the presence of alkaloids, sterols, and glycosides (Table 2). Figures 8a and 8b are showing the results of these tests. Additionally, the concentration of β-ODAP in the ten populations was also quantified using HPLC. Beta-ODAP (3-N-oxalyl-2,3-diaminopropionic acid) is a non-protein amino acid which is known to cause degeneration of motor neurons i.e., neurolathyrism in both human beings and animals. The content of Beta-ODAP in seed extract differed amongst populations, ranging from 1.04 to 315.2 (g/gm). The maximum level of this neurotoxin was detected in the seeds of Jammu plants (315.2 g/gm), while the lowest amount

(2.56g/mg) was found in the plants growing in Punjab state's Bani Lodhi. The contents of those from Sunderchak and Ratwana are also modest, at 4.73 g/gm and 5.75 g/gm, respectively.

Table 2. Qualitative analysis of the seeds showing the results of colour tests.

S.No	Bioactive compounds	JU	KA	RJ	BL	SD	RE	RW	NI	JH	RO
1.	Glycosides	+	+	+	+	+	+	+	+	+	+
2.	Phenols	-	-	-	-	-	-	-	-	-	-
3.	Flavonoids	-	-	-	-	-	-	-	-	-	-
4.	Alkaloids	+	+	+	+	+	+	+	+	+	+
5.	Sterols	+	+	+	+	+	+	+	+	+	+
6.	Anthocyanidins	-	-	-	-	-	-	-	-	-	-
7.	Saponins	-	-	-	-	-	-	-	-	-	-
8.	Phlobtanins	-	-	-	-	-	-	-	-	-	-

Fig.8a Results of the tests performed for the presence of various biochemicals (water extract)

Fig.8b Results of the tests performed for the presence of various biochemicals (methanolic extract)

Molecular studies: For molecular studies protocol for DNA isolation has been standardized (Fig.9). Further the isolated DNA from different populations is being analysed for genetic diversiy ststudies using ISSR markers.

Fig. 9 Bands of DNA observed during Gel-Electrophoresis

DEPARTMENT OF BOTANY

UNIVERSITY OF JAMMU

RUSA 2.0 (Strengthening Research Needs)

Utilization of the funds sanctioned vide Nos.RUSAJU/2/2019-20/111/3588-3636 dated: 14-11-2019 and RUSAJU/2/2019-20/111/4588 dated: 23-02-2021

STATEMENT OF EXPENDITURE

Name of the Faculty: Professor Veenu Kaul

Item of expenditure	Total grant approved and released by the RUSA (University of Jammu) after re-appropriation (Rs)	Total expenditure incurred (Rs)	Unspent Balance (Rs)
a)Hiring of services/ Honorarium for experts	65,000/-	36,580-00	28,420-00
b) Equipment (Repair) or any accessory, if needed, to the existing equipment	90,000/-	88,952.50	1047.50
c) Purchase of Minor equipment	1,00,000/-	98,918.00	1081.44
d) AMC's of the existing Equipment	----------		
e)Consumables/Chemicals/Glasswares	1,00,000/-	99,999-00	1-00
f) Contingency	50,000/-	49,923-00	77-00
g)Field Work	10,000/-	---------	10,000-00
h) Any other item after the approval of the Competent Authority	----------		
Total	4,15,000/-	3,74,372.50	40,626.94

Veenu Kaul 10/06/2021

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PI

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