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Potential wild rose germplasm of Western Himalayas—Conservation, evaluation and registration

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ABSTRACT

The untamed roses (*Rosa moschata, R. cathayensis*) growing in the Himalayan region of the country are unutilized and disappearing very fast due to various factors and need to be evaluated and conserved as important germplasm. Some distinct strains are expected from the natural population which may have potential to be utilized in the improvement of roses. Collection of distinct wild strains of roses was done for their domestication, evaluation, conservation and utilization on the basis of morphological characteristics, viz. colour, shape and size of the opening buds, flower type, i.e. single or double, size and vigour of the plants. Twenty one strains responded to the domestication process and were further evaluated for various traits. Significant variations were observed among the collected species and their strains for the evaluation parameters studied. Only four elite strains IHBT-WR-24, IHBT-WR-16, IHBT-WR-23 and IHBT-WR-21 belonging to four different species with desirable traits, viz. winter active growth, compatibility and flower production potential were identified in the present study as suitable wild rose germplasm. These strains were registered with Plant Germplasm Registration Committee, National Bureau of Plant Genetic Resources, New Delhi.

Key words : Collection, Conservation, Evaluation, Germplasm, Utilization, Wild roses

Wild roses are important sources of valuable germplasm for creating variability and improvement of roses as per the enormous future needs. The rose species growing wild in Indian Himalayan ranges at an altitude of 500 to 4700 meters (Hooker 1879, Bailey 1947 and 1976, Gupta 1979, Chowdhery and Wadhwa 1984, Collet 1984, Ambasta 1986, Sabnis 1986, Sharma and Jamwal 1988, Naithaini 1990, Pal 1991) are well adapted to the climatic conditions of the region and may have some distinct and potential strains which can play a significant role in the development of future roses. Wide range of evaluation studies with respect to the wild rose morphology, vigour, multiplication, compatibility to scion, winter activeness, quality flower shoot production and adaptability to various climatic variations are required in order to explore the value of wild rose strains as an important germplasm asset for the future which may be utilized as potential source for improvement in breeding programme or as rootstocks. Many important germplasm remain unutilized and become extinct in nature due to the various developmental and environmental factors as well as lack of prompt and perfect evaluation methods for their diverse properties and utilization. It is need of the hour to evaluate, conserve and utilize this valuable and rare germplasm existing in nature. Indian wild roses have not been exploited so far for their various uses, as in several Arabian and European countries where they are used in decoration, food, cosmetic, essential oil and pharmaceutical industries due to multi-purpose uses of the plant in form of ornamental flowers, nutraceutical products from fruits, essential oil, seed oil and pulp (Daels *et al.* 2002, Daysoylu *et al.* 2005, Ercisli and Guleryuz 2005, Musa Özcan 2002 and Uggla and Martinsson 2005).

Considering the various uses and future values of wild rose germplasm, a survey was conducted in the mid-hill altitudes (ranging from 800-1300 m amsl) of Kangra region of Western Himalayas (representing sub-tropical climatic conditions) for collection of distinct strains of wild roses for their domestication, evaluation, conservation and morphological studies. The collected strains were distinct in terms of various morphological characters and can be useful in various future research and development programmes in roses. Morphological variability present in wild strains of rose species has been reported earlier in *Rosa moschata* (Dhyani and Mukherjee 2002) and *R. cathayensis* (Dhyani 2004). Experimental findings of evaluation of winter active strains, compatibility and flower production potential studies are described in this research paper.

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MATERIALS AND METHODS

The collection, conservation and morphological variability studies were conducted at the Institute of Himalayan Bioresource Technology, Palampur (1320 m amsl 32º68´N, 76º38´E) situated in the mid hills of Himachal Pradesh. In this area the wild roses bear flowers during mid March to the end of May. Thus, it is the most suitable period for collection. A survey was conducted during this period in 1991-92 for collection of diversity present in the natural population and their domestication, evaluation, conservation and further utilization. Plants of surviving strains were multiplied gradually and different experiments were designed in phase wise manner over a period of time one after the other for evaluation and selection of suitable germplasm. Cytological studies of 4 selected germplasm were carried out during 2009-10. The research findings of many years on various evaluation parameters such as morphology, winter active, compatibility, flower production potential and cytology studies are described in this research paper.

Collection of distinct strains of roses was done on the basis of morphological characteristics, viz. colour, shape and size of the opening buds, flower type, i.e. single or double, size, and vigour of the plant. The morphological characters of the collected strains were recorded at the site of collection and later at the site of domestication. Stem cuttings (20 cm long) were collected from the selected plants for domestication and multiplication. These stem cuttings were planted in the field for rooting. After rainy season rooted cuttings of surviving strains were shifted in the germplasm plot. Data on morphological characteristics were recorded for further identification and evaluation studies. Based on the morphological and taxonomic studies, the surviving 21 strains were identified and they belong to 5 species, viz *R. brunonii*, *R. cathayensis*, *R. moschata*, *R. multiflora* and one strain similar to *R. alba*. These strains were compared to a well known rose rootstock *R. indica* var. *odorata* (IHBT-0) as the standard control in all the evaluation experiments.

The screening criteria for selection of valuable germplasm among the domesticated wild rose strains was based on four aspects, comprising evaluation for winter active growth, compatibility of strains to scion, suitability of grafting methods and seasons on compatibility and selection of clonal stocks based on performance expressed as the number and length of flowering shoots of combination plants. A series of experiments were conducted to evaluate the performance of germplasm collections of roses representing strains of different species collected from the wild. In the initial experiment, the strains were evaluated for winter active growth and compatibility to scion along with control. To determine winter active strains, data were recorded with respect to growth of the tagged shoots, at an interval of 15 days for a period of three months (15 November to 15 February) during the winter season. The data were recorded again next year during the said period in the same way. To select better compatible strains from the collected population of wild roses three methods of propagation, i.e. T-budding, stenting and cleft grafting have been tested over three seasons, i.e. spring, rainy and winter over a period of two years. Compatibility experiments were conducted on 15 stem cuttings (20 cm long) of each strain which were planted in propagation chamber for simultaneous rooting and sprouting of scion. Observations were recorded on % compatibility response in each strain using 'Scarlet Queen Elizabeth' as scion variety.

Prior to statistical analysis, the percentage data on % compatibility were transformed using square root transformation to satisfy the condition of homogeneity of variance. Data was pooled over two years and analysis of variance was done to work out the variations among the strains with three seasons as replicates, while the effect of seasons and method of grafting on compatibility of strains was analyzed using Student's t-test employing pooled standard deviations for the comparisons.

Based on the compatibility performance in the first experiment, the plants of seven strains, viz. IHBT-WR-21 (similar to *R. alba*), IHBT-WR-16 (*R. brunonii*), IHBT-WR-24 (*R. multiflora*), IHBT-WR-27, IHBT-WR-01, IHBT-WR-26 and IHBT-WR-23 (all *R. cathayensis*) were selected and shifted in the field for further evaluation of flower production potential in the second experiment. Observations were made on different grades of marketable flower shoot production (A > 60 cm; B = 45-60 cm; C = 30-45 cm) on four plants of each strain over four years. Data were also recorded on total number of flowering shoots, average height (cm) and average diameter (mm) of flowering shoots. The mean values were calculated for each strain and the variations between the strains were analyzed using F-test. The data over four years were used as replicates to assess the influence of the environment over the performance of the rose strains. Data of the experiment was utilized to determine genotypic, phenotypic and environmental variances and heritability estimates ($H = Vg/Vp \times 100$) for the different traits were calculated.

Four elite type strains having better or at par compatibility, winter activity and flower production potential compared to control were identified in the present study as suitable indigenous wild rose germplasm and being maintained *in situ* as mother stocks. These strains were registered with Plant Germplasm Registration Committee, National Bureau of Plant Genetic Resources (NBPGR), ICAR, New Delhi.

Cytological studies were carried out in the four potential strains to determine their ploidy level. Root tips from freshly rooted cuttings were collected for cytological analysis. The roots were pretreated with colchicine (0.01% aqueous solution) for 4 hr and fixed in Carnoy's fluid (1:3 acetic acid/ Absolute alcohol) at about 4°C for 24 hours. The fixed roots were hydrolyzed in 1N HCl at 60°C for 8-10 minutes,

Table 1 Description and mean performance of wild rose germplasm for winter active growth and compatibility pooled over two years

Strain No.	Species	Voucher specimen number	% winter active growth	% compa-tibility
IHBT-WR-01	*R. cathayensis*	PLP 9536	42.61	26.85
IHBT-WR-23	-do-	PLP 9535	44.68*	47.19*
IHBT-WR-27	-do-	PLP 9391	47.45*	28.69
IHBT-WR-26	-do-	PLP 9534	45.22*	44.43*
IHBT-WR-25	-do-	PLP 9565	31.40	23.14
IHBT-WR-03	*R. multiflora*	PLP 9562	12.82	15.74
IHBT-WR-04	-do-	PLP 9386	60.89*	19.43
IHBT-WR-18	-do-	PLP 9533	8.39	22.21
IHBT-WR-19	-do-	PLP 9390	1.37	9.26
IHBT-WR-24	-do-	PLP 9531	4.41	42.58*
IHBT-WR-10	*R. brunonii*	PLP 9388	4.51	3.70
IHBT-WR-15	-do-	PLP 9566	11.67	22.20
IHBT-WR-16	-do-	PLP 9530	24.81	29.62
IHBT-WR-17	-do-	PLP 9567	2.16	15.74
IHBT-WR-06	*R. moschata*	PLP 9549	40.30	0.00
IHBT-WR-08	-do-	PLP 9387	14.36	2.78
IHBT-WR-11	-do-	PLP 9561	10.32	3.70
IHBT-WR-13	-do-	PLP 9389	16.35	0.00
IHBT-WR-14	-do-	PLP 9563	7.99	1.85
IHBT-WR-22	-do-	PLP 9564	13.86	11.11
IHBT-WR-21	Similar to *R. alba*	PLP 9532	53.46*	23.14
IHBT-0	*R. indica* var. *odorata*		2.45	24.99
Overall mean			22.79	19.01

*Significant at P=0.05

followed by staining with 2% aceto-carmine and then squashed for cytological observations. The slides were examined using a Nikon biological research photomicroscope (Model: 80i) equipped with digital camera and good preparations photographed and prints made from which the metaphase chromosomes of five cells were counted.

RESULTS AND DISCUSSION

Performance of different strains for their winter active growth and compatibility to scion is presented in Table 1. In the first experiment, the overall mean response of wild rose strains to winter active growth during winter months was 22.79% and ranged from 1.37% to 60.89% compared to 2.45% response of the control, Strain No. IHBT-0 (average max./min. temperature during observation months of the study period was 17.57/7.33°C). Based on analysis of variance of the data significant variation was recorded for winter active growth among the wild rose strains with highest response in IHBT-WR-04 (*R. multiflora*) followed by IHBT-WR-21 (similar to *R. alba*), IHBT-WR-27, IHBT-WR-26 and IHBT-WR-23 (*R. cathayensis*), respectively (Table 2). A number of other strains had winter active growth at par with control.

Overall results pertaining to winter active growth suggest genotypic variations for this trait. Rootstocks required for flower production should be easily manageable clonal stocks having good vigour, winter activity and resistant to various stress including continuous harvesting. A rootstock can influence various aspects of the scion in terms of growth and development. Important parameters are adaptation to pH values of the soil, salinity tolerance, climatic factors such as frost resistance or winter hardiness, disease resistance, compatibility, plant longevity, vigour, productivity and flower quality. Winter active growth in roses is important trait which can produce flowering shoots even during winter season due to active growth in rootstocks and can enhance flower production ultimately. The winter active strains identified in the present study have significantly high winter activity during the observation period in comparison to control and may have potential use as clonal stocks. De Vries and Dubois (1997) observed significant correlations between early root growth during spring season and flower yield of clonal rose rootstocks. The active shoot growth observed during winter season may be due to root growth during this period suggesting better prospects for increased flower yield of such strains.

It was observed in this study that vegetative growth of various strains cease as winter advanced during the observation period and in some strains tip burning and drying was noticed

Table 2 Pooled analysis of variance for response to compatibility and winter active growth of wild rose strains

Source of variations	df	% winter active growth		df	% compatibility	
		Mean square values	F-Ratio		Mean square values	F-Ratio
Replications (within years)	6	1411.94	4.51*	4	20.97	5.65*
Years	1	745.81	2.38	1	5.62	1.52
Strains	21	2943.51	9.40*	21	20.49	5.52*
Interaction	21	637.14	2.03*	21	1.32	0.32
Error	126	313.25		84	3.71	

*Significant at P=0.05

due to frost injuries and low temperature. Fresh growth was observed in all the strains after March when the temperature increased. However, it was also observed that many strains which have good winter active growth did not respond equally well to compatibility with scion and it may be due to the genetic make up of individual strains. Species-wise, a number of *R. cathayensis* strains had good winter active growth, whereas most of the *R. moschata* strains had poor growth during winter season. Significant genotypic variation for the trait in different species suggests that the trait may be improved through a breeding program involving contrasting genotypes as parents.

Scion-rootstock compatibility studies in roses highlight the variations in the vigour of the rose combination plants resulting from variations in the individual vigour of scion varieties and rootstocks. Rootstocks enable relatively fast and cheap multiplication of large number of scion varieties, give combination plants a good hold in the soil, control plant vigour, guarantee a good uptake of water and nutrients and bring about improved winter survival. Vigour of garden / cut-rose varieties on various stocks is commonly expressed as the number or length of shoots of combination plants in the nursery. Compared to control (*R. indica* var. *odorata*) which is the most commonly used rootstock, there are number of non-recurrent flowering diploid rose species which have potential use as clonal stocks. In the current study we present data about the performance of potential clonal stocks based on selection and propagation of desirable genotypes from the wild.

Regarding compatibility, the overall response of wild rose strains was 19.01% and ranged from 0 to 47.19% (Table 1) as compared to 24.99% response of the control. Based on analysis of variance of the transformed data significant variation was recorded among the strains studied and highest response to compatibility with scion variety 'Scarlet Queen Elizabeth' was obtained for IHBT-WR-23 followed by IHBT-WR-26 (both *R. cathayensis*) and IHBT-WR-24 (*R. multiflora*) (Table 2). On an overall basis, high genotypic variability was observed for scion-stock compatibility. Two of the strains IHBT-WR-06 and IHBT-WR-13 failed to respond in terms of compatibility. The observation conforms to the view that any rose may be used as a stock, but not all stocks may prosper alike (De Vries 2003).

Significant variations were also observed for the replications (Table 2) which pertain to seasonal effects on the response to compatibility of the strains. Based on t-test, effect of seasons on response to compatibility of wild rose strains was worked out and highest response was observed in December followed by March and July (Table 3). Response to compatibility in December was significantly greater than that of July month but was at par to compatibility in the month of March. Regarding the effect of propagation method on response of strains to compatibility with scion, budding was the most efficient method closely followed by stenting and in this experiment both these techniques were observed to be significantly superior to cleft grafting (Table 4). Efficiency of grafting methods over different seasons exhibit significant and highest response to budding in December and March followed by stenting in July and December and cleft grafting in December (Table 5).

Table 3 Effect of seasons on response of wild rose strains to % compatibility

	July/ December	July/ March	December/March
X1	6.90	6.90	12.95
X2	12.95	8.83	8.83
Difference	6.05	1.93	4.12
Pooled SD	6.72	5.75	6.71
t (calculated)	2.99*	1.11	2.03
t (tabulated)	2.08	2.08	2.08

*Significant at P=0.05; X1, Mean performance of 22 strains in the first season; X2, Mean performance of 22 strains in the second season

In the second experiment mean data for all the traits, viz. number of flowering shoots, number of grade A shoots, number of grade B shoots and height of flowering shoots is presented in Table 6. Based on analysis of variance of data over four years, significant variations were observed among strains for number of flowering shoots, number of grade A shoots, number of grade B shoots and height of flowering shoots (Table 7). Significant variations for most of the traits were also observed over years. Strain IHBT-WR-24 is significantly superior, whereas IHBT-WR-27 and IHBT-WR-1 are significantly inferior to the control (15.65) for number of flowering shoots. In case of grade A shoots, strain IHBT-WR-24 was significantly superior whereas, IHBT-WR-27 and IHBT-WR-1 were significantly inferior to the control (6.93). Regarding grade B shoots, strains IHBT-WR-24 and IHBT-WR-16 were at par with the control (4.43). In case of average height of flowering shoot IHBT-WR-24 was at par with the control (62.3 cm) but significantly superior to strains IHBT-WR-21, IHBT-WR-16, IHBT-WR-27 and IHBT-WR-

Table 4 Effect of methods on response of wild rose strains to % compatibility

	Stenting/Cleft grafting	Stenting/Budding	Cleft grafting/ Budding
X1	10.10	10.10	6.73
X2	6.73	11.82	11.82
Difference	3.36	1.72	5.09
Pooled SD	5.04	6.05	5.44
t (calculated)	2.21*	0.94	3.10*
t (tabulated)	2.08	2.08	2.08

*Significant at P=0.05; X1, Mean performance of 22 strains in the first method; X2, Mean performance of 22 strains in the second method

Table 5 Comparison of methods over seasons for response to % compatibility

	Stenting			Cleft grafting			Budding		
	July/ Dec.	July/ March	Dec./March	July/ Dec.	July/ March	Dec./March	July/ Dec.	July/ March	Dec./March
X1	3.96	3.96	3.88	1.92	1.92	3.34	1.02	1.02	5.73
X2	3.88	2.26	2.26	3.34	1.48	1.48	5.73	5.08	5.08
Difference	0.07	1.69	1.62	1.42	0.43	1.85	4.71	4.06	0.64
Pooled SD	3.06	2.58	2.44	2.66	2.22	2.16	2.30	2.20	3.49
t (calculated)	0.08	2.17*	2.20*	1.76	0.64	2.84*	6.77*	6.11*	0.61
t (tabulated)	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08

*Significant at P=0.05; X1, Mean performance of 22 strains in the first season; X2, Mean performance of 22 strains in the second season

Table 6 Mean values of rose strains for different traits (Pooled over four years)

Strain No.	Total number of flowering shoots	Number of grade A (> 60 cm) shoots	Number of grade B (45-60 cm) shoots	Number of grade C (30-45 cm) shoots	Average height (cm)	Average diameter (mm)
IHBT-WR-21	14.62	6.28	4.20	3.74	62.73	5.96
IHBT-WR-16	18.24	7.53	5.24	4.49	60.14	5.85
IHBT-WR-24	18.74*	9.74*	5.34	3.71	66.91	6.39
IHBT-WR-27	11.25	4.31	3.43	3.37	55.41	5.43
IHBT-WR-1	10.9	4.62	3.43	2.93	58.20	5.50
IHBT-WR-26	13.85	5.8	4.65	3.45	60.33	6.83
IHBT-WR-23	13.67	7.35	3.57	2.72	64.76	6.27
IHBT-0	15.65	6.93	4.43	4.24	62.3	6.04
μ	14.61	6.57	4.29	3.58	61.35	6.03
Critical difference	2.6	1.6	1.37	1.36	6.30	1.10

*Significantly superior; P≤ 0.05

Table 7 Analysis of variance for different morphological traits among selected strains of roses

Source of variations	Degree of freedom	Mean of squares					
		Total number of flowering shoots	Number of grade A (> 60 cm) shoots	Number of grade B (45-60 cm) shoots	Number of grade C (30-45 cm) shoots	Average height (cm)	Average diameter (mm)
Years	3	32.71*	12.17*	2.36*	1.44	53.19*	0.86
Strains	7	142.72*	99.99*	11.27*	26.25*	627.94*	3.25*
Error	21	3.12	1.19	0.89	0.86	18.39	0.56
H (%)		97.81	98.81	92.10	96.72	97.07	82.76

P ≤ 0.05

1. The overall mean values of all the traits studied and corresponding performance of IHBT-0 were observed to be at par. High heritability estimates were observed for the traits in this experiment (Table 7).

Earlier studies by De Vries and Dubois (1997) and Ximing (2002) support the outcome of the second experiment regarding pronounced differences among the stocks for flower shoot yield. Clonal stocks have been selected from *R. multiflora* and *R. multiflora* var. *cathayensis* population at Wageningen University in the past (De Vries 2003). The present study confirms the potential of selected *R. multiflora* and *R. cathayensis* strains as suitable stocks for scion-stock studies and breeding for new rootstocks. High heritability estimates for the different parameters studied suggests the potential of the strains for use as parents in the breeding program for improvement of the traits of interest.

Registration of wild rose germplasm

The selected strains of wild roses have potential to be utilized as rose rootstocks, and in the improvement of roses and rootstocks through breeding. Four domesticated and potential accessions of IHBT are well adapted to the region

Table 8 Characteristics of registered rose germplasm

Rose accessions	National Identity	Registration Numbers	Characteristics
IHBT-WR-24 (*R. multiflora*)	IC549905	INGR08066	Flower white double, diameter 5.64 cm, flowers/panicle 54.2 and number of petals/ flower 29.2, Sepals and thalamus hairy, No. of hips/panicle 18.8, size of hips 0.73 cm, No. of seeds/hip 10.3. Easy to multiply and produce better number and height of flowering shoot than the control.
IHBT-WR-16 (*R. brunonii*)	IC549906	INGR08067	Flower white, single, diameter 5.31 cm, No. of flowers/panicle 44.7 and number of petals/ flower 5-9, Sepals and thalamus glandular, Number of hips/panicle 34.80, size of hips 0.73 cm, number of seeds/hip 11.2.Vigorous, compatible to scion and produced better number and height of flowering shoots than the control.
IHBT-WR-23 (*R. cathayensis*)	IC549907	INGR08068	Flowers double, pink colour, diameter 4.16 cm, number of petals/ flower 28, number of flowers/panicle 32, Sepals hairy and glandular, thalamus hairy, number of hips/panicle 1, size of hips 0.6 cm, number of seeds/hip 7.0.Winter active, easy to multiply, better compatible to scion, and produced number and height of flowering shoots at par to the control.
IHBT-WR-21 (similar to *R. alba*)	IC549908	INGR08069	Flower white with pinkish tinge in early stage, double, diameter 4.16 cm, number of flowers/panicle 16.7 and number of petals/ flower 75, Sepals and thalamus hairy, Number of hips/panicle 4.1, size of hips 0. 6 cm, number of seeds/hip 7.0.Winter active, easy to multiply, compatible to scion, and produced number and height of flowering shoots at par to the control.

(Standard rootstock *R. indica* var. *odorata* was used as control)

and represent new germplasm which have been awarded registration and national identity numbers by the Plant Germplasm Registration Committee, NBPGR, New Delhi in its XVII meeting on 16 February 2008. The details of rose accessions are presented in the Table 8 and Fig 1.

Cytological studies of the four potential strains IHBT-WR-24, IHBT-WR-16, IHBT-WR-23 and IHBT-WR-21 determined that the strains have diploid chromosome count (2n=2x=14) (Fig 2). Earlier studies on ploidy level as reported by Gudin (2000) confirm three of the species, viz *R. multiflora*, *R. brunonii* and *R. cathayensis* (Rehder 1947, Darlington and Wylie 1955, Maia and Venard 1976) having diploid chromosome number (2n=2x=14), while the chromosome count of *R. alba* is reported to be 2n=6x=42 (hexaploid). However, morphological, taxonomic and cytological observations in the present study suggest that IHBT-WR-21 is a distinct species at diploid level similar to *R. alba*. Interestingly only a single strain similar to *R. alba* was identified during germplasm collection and no other related strain has been observed in the collection site.

In conclusion the studies indicate that there are potential germplasm present in the natural population and need to be conserved after thorough evaluation. The degree of variability within the different *Rosa* species for most of the parameters studied was found to be significant particularly with respect to response to domestication process, winter active growth and compatibility. Selection of desirable germplasm from wild cannot be done directly from the natural growing areas where huge population occur, because there is no methodology or process developed so far which can differentiate the potential germplasm from others unless and until they are tested for various parameters at the site of domestication over a period of time. The results of the study led to

Fig. 1 Flower images of registered germplasm (Germplasm registration number/ National Identity Number in parentheses): a) IHBT- WR-24 (INGR 08066/IC549905); b) IHBT- WR-16 (INGR 08067/IC549906); c) IHBT- WR-23 (INGR 08068/IC549907); d) IHBT- WR-21 (INGR 08069/ IC549908).

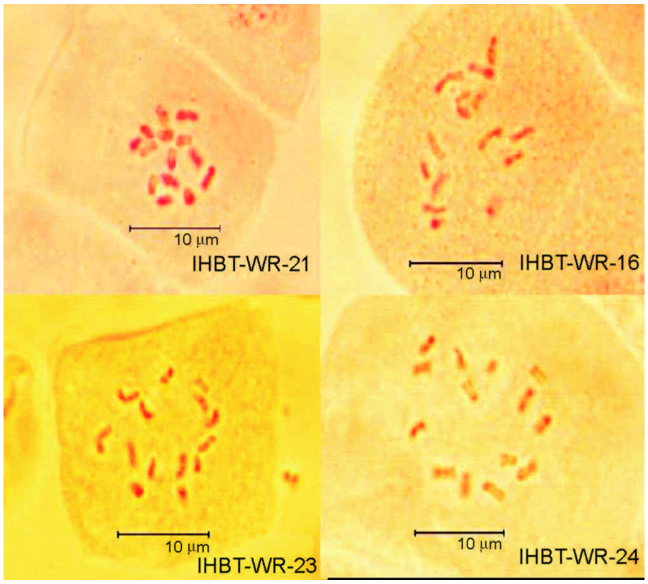

Fig 2 Chromosome count (2n = 2x = 14) of the four potential strains

registration of potential germplasm resources of wild roses of the west Himalayan region and suggests that evaluation studies involving wild resources is a time consuming process requiring long drawn strategies for their collection, domestication, evaluation and utilization in future.

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