BIOLOGIA PLANTARUM (PRAHA)

7 (1) : 7—12, 1965

Hybrid between Tetraploid *Phaseolus* sp. and *P. calcaratus* Roxb.

S. Dana

Department of Cytogenetics and Plant Breeding, College of Agriculture,

Kalyani University, Kalyani, West Bengal, India

Received February 17, 1964

Abstract. 1. In tetraploid *Phaseolus* sp. × *P. calcaratus* cross, shrivelled but viable seeds were produced. One hybrid was raised but it died within a month. Another hybrid was raised to flowering by artificially culturing the embryos before shrivelling.

2. The F_1 of tetraploid *Phaseolus* sp. × *P. calcaratus* cross, had various leaf abnormalities at an early stage. In most of the characters the hybrid was intermediate between the two parents. The twining habit of *P. calcaratus* dominated in the hybrid. The F_1 plant had 10·7 per cent pollen fertility. The hybrid died within a week of flowering.

3. In *P. calcaratus* × tetraploid *Phaseolus* sp. cross, rudimentary, non-viable seeds were produced.

4. The incompatibility barriers are active — (a) at post-fertilization stages causing shrivelled and rudimentary seeds and (b) in the F_1 generation causing hybrid lethality and sterility. The importance of embryo culture techniques in removal of the first type of crossability barriers is stressed.

The tetraploid *Phaseolus* sp. is a good fodder crop. But it has a long span of flowering and pod formation which is not conducive to economic seed production. On the other hand, *P. calcaratus* has a short blossoming period which makes seed harvest an easy process due to uniform maturity of pods. This cross was undertaken to transfer the short blossoming period to the tetraploid *Phaseolus* sp. Interspecific crosses in *Phaseolus* have been reviewed previously by Sen and Ghosh (1960) and Dana (in press, a).

Material and Methods

Seeds of tetraploid *Phaseolus* sp. and *P. calcaratus* Roxb. were collected through the courtesy of Fodder Botanist, Haringhata, West Bengal, form cultivated plants. The tetraploid *Phaseolus* sp. is yet to be identified. A detailed description of this species is given by Dana (1964). This species has 2n = 44 and *P. calcaratus* has 2n = 22 chromosomes.

Hybridization technique and the method of embryo culture were already described in another paper (DANA 1964).

The colour of floral parts was compared with the illustrations in "A Dictionary of Colour" (MAERZ and PAUL, 1950).

Results

Crossability:

Mature pods were obtained from both directions of this cross. When *P. calcaratus* was used as female parent, the mature pods contained few rudimentary non-viable seeds. Some very small shrunken but viable seeds were obtained with tetraploid *Phaseolus* sp. as female parent. One seedling was raised from these seeds but it died within a month. It was stunted in growth and had poor root system. Table I contains these results.

Raising of hybrid through embryo culture:

Table 1

Results of hybridization and germination of hybrid seeds

		Crosses	
Female parent		4 n *Phaseolus* sp.	*P. calcaratus*
Male parent		*P. calcaratus*	4 n *Phaseolus* sp.
No. of flowers pollinated		68	107
% of pods at different days after pollination and at harvest	1	98·5	96·3
	4	70·5	76·6
	7	52·9	55·1
	14	45·6	6·5
	At harvest	39·7	4·7
Seeds obtained	Total	170	24
	Shrunken (in %)	48·8	0
	Rudimentary (in %)	51·2	100
No. of seeds soaked		83	24
Germination percentage		6·0	0
Survival % of seedlings	At 7 days	1·2	0
	At flowering	0	0

When tetraploid *Phaseolus* sp. was used as female parent, some mature pods with a few viable seeds were obtained. But it was observed that the pods started withering 3 weeks after pollination and most of the seeds collapsed during this stage. Embryos (4·3 mm. long) were dissected out of the seeds at the age of 19 to 20 days. Ten dissected embryos were cultured in modified SANDERS at al. (1959) agar medium, of which 7 were found to be growing after 4 days of incubation and later on, all of them were transplanted to soil.

The hybrid seedlings raised by embryo culture were slow growing at early stages and had various types of leaf abnormalities. Often the first few leaves were unifoliate.

Sometimes, two or more adjacent leaves were fused together forming a complex structure. All seedlings except one died at different stages up to the age of 2 months. The surviving plant was vigorous. The hybrid was slightly affected during heavy rains while the *P. calcaratus* parent succumbed.

At early stages, the hybrid had erect growth habit like the tetraploid *Phaseolus* parent, but later on it developed twining branches like those of *P. calcaratus*. The hybrid died within a week of flowering.

In the hybrid, the dominating characters of tetraploid *Phaseolus* sp. were — nature of leaf margin, colour of stipule, colour of stem, and early growth habit.

Characters of *P. calcaratus* dominated in the hybrid in the shape of stipule and in later growth habit.

The colour of floral parts in tetraploid *Phaseolus* sp. and in *P. calcaratus* were very similar and they had overlapped in the colour of floral parts in the hybrid.

The characters of the parents and the hybrid are compared in Table 2.

Discussion

The incompatibility barriers are active at post-fertilization stages of the cross in causing disharmony between the hybrid embryo and the surrounding tissues. This is shown by the shrivelled seeds in tetraploid *Phaseolus* sp. × *P. calcaratus* cross and non-viable rudimentary seeds in the reciprocal combination. But this crossability barrier was easily surmounted with the aid of embryo culture and the mature hybrid has been raised from the tetraploid *Phaseolus* sp. × *P. calcaratus* cross.

Embryo culture techniques helped to remove this type of crossability barrier in many other crosses as in *Linum austriacum* × *L. perenne* (LAIBACH 1925), in *Melilotus officinalis* × *M. alba* (WEBSTER 1955), in *Phaseolus vulgaris* × *P. acutifolius* (HONMA 1955) and in tetraploid *Phaseolus* sp. × *P. ricciardianus* (DANA 1964).

The incompatibility barriers were also very effective in the F_1 generation through hybrid lethality. At early stages of growth hybrid lethality was quite common but even the mature plant was not beyond its reach. The sterility in F_1 was also a manifestation of the action of incompatibility barriers.

The present cross succeeded only with the tetraploid *Phaseolus* sp. as female

Table 2

Comparison of contrasting characters of parents and hybrid

Characters	Female parent 4 n *Phaseolus* sp.	Hybrid	Male parent *P. calcaratus*
Stem — a) Colour	Deep purple	Deep purple	Light purple
— b) Habit	Main shoot erect; branches suberect	Main shoot erect; branches twining	Bushy with twining branches
Mature trifoliate leaf			
a) Margin of leaflets	Entire	Entire	Terminal — entire lateral — slightly lobed
b) Colour of stipule	Light purple spotted	Light purple spotted	Green
c) Shape of stipel	Ovate	Linear	Linear
Flower			
a) Size (in mm.)	14·1 ± 0·30	19·0 ± 0·19	11·0 ± 0·26
Standard			
a) Colour	Golden Rod	Golden glow	Aureolin P.
b) Size (in mm.)	24·0 ± 0·20 × 17·2 ± 0·26	21·2 ± 0·23 × 15·8 ± 0·13	18·4 ± 0·19 × 13·0 ± 0·11
Wing			
a) Colour	Golden yellow	Ta-Ming	Golden glow
b) Size (in mm.)	14·6 ± 0·29 × 11·4 ± 0·30	14·0 + 0·34 × 10·9 ± 0·22	16·5 ± 0·19 × 6·5 ± 0·12
% **of stainable pollen**	98·8	10·7	96·6
Length of style (in mm.)	21·9 ± 0·23	19·6 ± 0·28	17·9 ± 0·22

parent. The difference in chromosome numbers of the two parental species may be the cause of unidirectional crossability.

Interspecific hybridization frequently disturbs the balanced actions of different genes (STEBBINS 1958, p. 155). In the present cross, this was shown in leaf abnormalities of the F_1 seedlings.

The twining habit of *P. ricciardianus* dominated in the F_1 even in the presence of two genomes from the tetraploid *Phaseolus* sp. The dominance of twining habit may be due either to the primitive nature of this character or to the possible interaction of the three genomes in the hybrid. Similar features were observed in the tetraploid *Phaseolus* sp. × *P. ricciardianus* cross (DANA 1964).

References

DANA, S.: Cross between *Phaseolus aureus* ROXB. and *P. mungo* L. (in press, a).

DANA, S.: Interspecific cross between tetraploid *Phaseolus* species and *P. ricciardianus* TEN. Nucleus **7** (1) : 1—10, 1964.

HONMA, S.: A technique for artificial culturing of bean embryos. — Proc. Amer. Soc. Horti. Sci. **65** : 405—408, 1955.

LAIBACH, F.: Das Taubwerden von Bastardsamen und die künstliche Aufzucht früh absterbender Bastardembryonen. — Zeitschr. Bot. **17** : 417—450, 1925.

MAERZ, A. and PAUL, M. R.: A Dictionary of Colour. — Mc Graw Hill Book Co. Inc. New York. 2nd ed. VII and 208 p., 1950.

SANDERS, M. E. et al.: Influence of environmental factors on origin of colchicine induced true breeding diploid mutants in *Sorghum*. — Amer. J. Bot. **46** : 119—125, 1959.

SEN, N. K. and GHOSH, A. K.: Interspecific hybridization between *Phaseolus aureus* ROXB. (Green gram) and *Ph. mungo* L. (Black gram). — Bull. Bot. Soc. Bengal. **14** : 1—4, 1960.

STEBBINS, G. L.: The inviability, weakness and sterility of interspecific hybrids. — Adv. Genet. **9** : 147—215, 1958.

WEBSTER, G. T.: Interspecific hybridization of *Melilotus alba* × *M. officinalis* using embryo culture. — Agron. J. **47** : 138—142, 1955.

S. DANA, odd. cytogenetiky a šlechtění rostlin, zemědělská fakulta, Kalyanská universita, Kalyani, záp. Bengálsko, Indie: **Hybrid mezi tetraploidními *Phaseolus* sp. a *P. calcaratus* ROXB.** —Biol. Plant. **7** : 7—12, 1965.

1. Při křížení tetraploidního druhu *Phaseolus* sp. × *P. calcaratus* vznikla scvrklá, ale životná semena. Jeden vypěstovaný kříženec během měsíce uhynul. Jiný byl dopěstován do květu umělou kulturou embrya před scvrknutím.

2. F_1 pokolení kříženec tetraploidního druhu *Phaseolus* sp. × *P. calcaratus* se vyznačovalo různými listovými abnormitami na raném stadiu. Ve většině znaků byl hybrid intermediérní. Dominoval u něho popínavý habitus *P. calcaratus*. Pylová fertilita F_1 rostliny byla 10,7 %. Hybrid týden po kvetení zašel.

3. Při křížení *P. calcaratus* × tetraploidní druh *Phaseolus* sp. vznikla rudimentární, neživotná semena.

4. Inkompatibilní bariéry působí:

a) po oplození, kde jsou příčinou svraskalých a rudimentárních semen

b) v F_1 generaci, kde jsou příčinou lethality a sterility hybridů. Zdůrazňuje se důležitost umělé kultivace embryí pro odstranění prvního typu inkompatibilní bariéry.

С. ДАНА, отдел цитогенетики и селекции растений, Сельскохозяйственный коллидж, Кальянский университет, Кальян, Западная Бенгалия, Индия: **Гибрид между тетраплоидным *Phaseolus* sp. и *P. calcaractus* ROXB.** — Biol. Plant. **7** : 7—12, 1965.

В скрещивании тетраплоидного вида *Phaseolus* sp. × *P. calcaratus* получены морщинистые, однако жизнеспособные семена. Один гибрид был высажен, однако, погиб в те-

чение месяца. Другой гибрид был доведен до цветения искусственной культурой зародыша до сморщения. F_1-поколение от скрещивания тетраплоидного вида *Phaseolus* sp. × *P. calcaratus* отличалось разными ненормальностями листьев на ранней стадии. В большинстве признаков гибрид был промежуточным между обоими родителями. Обвивающий характер *P. calcaratus* в гибриде доминировал. Растение F_1 имело 10,7 % фертильности пыльцы. Гибрид погиб в течение недели после цветения. В скрещивании *P. calcaratus* × тетраплоидный вид были получены рудиментарные, нежизнеспособные семена. Успех одного направления данного межвидового скрещивания обусловлен различиями хромосомных чисел родителей. Барьера нескрещиваемости — (а) на стадиях после оплодотворения обуславливает морщинистые и рудиментарными семена и (б) в F_1 поколении обуславливает летальность и стерильность гибридов. Использованием культуры зародыша возможно преодолеть первый тип барьеры нескрещиваемости.