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Use of Plant Growth Regulators to Enhance the Pod Setting in Wide Hybridization between Grasspea and Pea

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ABSTRACT: Wide crossing or distant hybridization has been used in the genetic improvement of crop plants by developing useful variability for breeding populations in crop. Distant crosses are more successful in more closely related species or genera than in less closely related species or genera. In distant crosses seed setting show very less and main problem depicted flowers and pods shading one or two days after pollination due to incompatibility reaction between both parents. To overcome flower and pod shading with increase seed setting use of plant growth regulator is one of the best option to overcome the problem in wide hybridization. From out of total 24 parents 18 grasspea genotypes and 7 pea genotypes were used under study. The effect of growth regulators with a combination of GA with NAA and Kinetin on pollinated buds peduncle showed the best result as compare to the other possibilities for both the parents grasspea and pea. The daily application of a mixture of growth regulators containing GA + NAA + kinetin to buds immediately after cross pollination and to the developing pods, considerably helped to retain a larger number of pods. The control condition retained upto 15 days with 3.36%, while the hormone-treated pods increased upto 8.76%. The maximum six seeds obtained by interspecific cross *Lathyrus odoratus* × IC 142855 followed by *Lathyrus odoratus* × Ratan and *Lathyrus odoratus* × IG 64975 with each of 4 seeds. Out of seven crosses only four crosses produce viable seeds in which *Lathyrus odoratus* × IG 64975 gave 4 viable and germinated seeds followed by *Lathyrus odoratus* × Ratan and *Lathyrus odoratus* × RGRLS 212-2 with 3 germinated seeds It shows that grasspea species are less compatible with other related species. In other hand for intergeneric crosses 225 seeds were germinated from 299 set seeds and 74 seeds nonviabile and immature seeds due to embryo abortion. The maximum 20 seed obtained from the cross Ratan × Indira Matar-1 in F_1 generation followed by 19 seeds in cross Paras × Mahateora shows the compatibility of both the parents used in those intergeneric crosses. In case of germination of seeds 17 germinated seeds in cross Ratan × Indira Matar-1 followed by Paras × Mahateora with 11 germinated seeds. The setting of seeds and its development depend on the application of Plant growth regulator.

Keywords: Plant growth regulator, grasspea, interspecific crosses, intergeneric crosses.

INTRODUCTION

Grasspea belongs to genus *Lathyrus*, within the *Fabaceae* family (syn. Leguminosae), subfamily *Faboideae* (syn. Papilionoideae), tribe *Fabeae* (syn. *Vicieae*), along with genera *Pisum*, *Vicia*, *Lens* and *Vavilovia* (Smykal *et al*., 2011). In early domestication being normally found with early domestication of pea (*Pisum sativum* L.), *Lathyrus sativus* is a derived from *Lathyrus cicera*, which is genetically nearest wild species of grasspea. *Lathyrus sativus* also have potential as sources of variation for closely related important legumes such as pea (*Pisum sativum*) and although they are cross incompatible (Bhardwaj *et al.*, 2020). Other economically important species grown commercially are the forage crop chickling vetch (*Lathyrus cicera*) and the ornamental sweet pea (*Lathyrus odoratus*). *Lathyrus cicera* has been cultivated since ancient times and was domesticated in Southern France and the Iberian Peninsula (Kislev, 1989) used as animal feed (White *et al.*, 2002) and *Lathyrus odoratus* originates from Southern Italy and has become an economically important ornamental plant grown for its cut flowers and for garden decoration.

Distant crosses are more successful in more closely related species or genera than in less closely related species or genera for transferring desirable genes like resistance against biotic or abiotic stress within varying environments, from related wild or cultivated species and genera into cultivated plants. It is an important research approach for valuable traits resistance composition of metabolites, morphological traits and their responsible genes are often found only within wild species (treasure of valuable genes), related species and genera of the cultivars but these are lacking in cultivated species mostly. So it helps in assemble all desirable traits in one species or nucleus. Like other methods there are also problems associated with this method, but after overcoming those problems it is possible to generate desirable hybrids. But the main drawback of distant crossing that embryo rescue and post fertilization barrier causes the very negligible percentage of success and seed setting. Sometimes shriveled seeds immature seeds developed and in some cases seeds developed but not germinated.

Globally, the area under grass pea cultivation is estimated at 1.50 million ha, with annual production of 1.20 million tonnes (Kumar *et al*., 2011). It has an area of 0.9 million ha with an annual production of 0.47 million tonnes in the country. In world 95.98 metric tonnes production, 23.24 metric tonnes in India which contributing around 24.21 percentage share with first rank of the total pulse production (Annon. 2019). The pulse area coverage in year 2018-19 in Chhattisgarh 0.74 million ha contributes 2.56 percent of country whereas for production Chhattisgarh 0.54 million tonnes contributes 2.29 percent production in the country with yield of 722 Kg/ha. In India are Bihar, Madhya Pradesh, Maharashtra, West Bengal and Chhattisgarh. Other States of Uttar Pradesh, Rajasthan, Gujarat, Andhra Pradesh and Karnataka are major grass pea growing States with limited quantity. People from lower economic sections consumed this legume crop as a staple food during famines and floods.

Grass pea can serve a variety of purposes, such as animal feed and fodder, but also as human food, thanks to 18–34% and 17% of protein content in seeds and mature leaves, respectively (Rizvi *et al.,* 2016). These values are higher than those of field pea (*Pisum sativum* sub sp. *Arvense* (L.) Asch) (23%), faba bean (*Vicia faba*) (24%), or lupine (*Lupinus albus*) (32%) (Petterson *et al.*, 1997), whereas animal feed from grass pea usually consists of ground or split grain or flour and is used primarily to feed lactating cattle or other draft animals, human diets include grasspea as grains that are boiled and then either consumed whole or processed for split dal (Enneking, 2011).

MATERIALS AND METHODS

From the total 24 parents 18 grasspea genotypes and 7 pea genotypes were used under study. The 3 grasspea popular varieties having high yield with low ODAP content used with 11 low ODAP germplasm accessions on the basis of catalogue data as compared to rest of the 1875 germplasm published by IGKV Raipur.

The experimentation was conducted during 2017-18 at Research cum Instructional farm of Indira Gandhi Krishi Vishwavidyalaya Raipur (Chhattisgarh). All 24 parent population with 2 replications sown in 2m length with 30 cm and 10cm for row to row and plant to plant distance respectively. These two replications were sown at 1 week time interval staggered sowing to maximum number of possible crossing could be done with constructive and desirable microenvironment for crossing. At time of flowering emasculation was done previous day of crossing and next day pollinate the bud with desirable genotype pollen dusting.

The flower buds of three species of grasspea viz., *Lathyrus sativus* L. *Lathyrus odoratus* and *Lathyrus cicera* those expected to blossom in the next morning and tagged all the flower buds with cross name and date of emasculation and pollination. In next morning pollination occurred with pollen grains dehiscence with the help of soft brush and scalpel of pea. One of the major problem in intergeneric and interspecific crosses flower drop and early abortion of embryo after pollination. To prevent early abortion of the embryo and to encourage the development of pods, use a solution that containing gibberellic acid (GA- 80 to 100 mg/l), naphthaleneacetic acid (NAA-20 to 25 mg/l) and kinetin (3 to 5 mg/l) with distilled water and applied to the base of the pedicel twice a day for 10 days after pollination.

RESULTS AND DISCUSSION

Effect of growth regulators on avoidance of flower drop and early embryo abortion. The daily application of the solution of a mixture of growth regulators containing GA + NAA + kinetin to buds immediately after cross pollination, and to the developing pods, considerably helped to retain a larger number of them longer.

The effect of growth regulator mainly depend on the different doses of plant growth regulators while it also affected by the time and stage of buds at the time of application. The effect of growth regulators with application of (GA 100 mg/l) + NAA (25 mg/l) + Kin (5 mg/l) on pollinated buds peduncle showed the best result as compare to the other possible combinations for retention and emerging pods in a cross involving different species of grasspea and pea. Effect of growth regulators on pod retention.

The daily application of the solution of a mixture of growth regulators containing GA + NAA + kinetin to buds immediately after cross pollination, and to the developing pods, considerably helped to retain a larger number of them longer (Mishra *et al.*, 2021). Whereas in the case of control only 3.36% of the pods were retained after 15 days, in the hormone-treated ones it increased to 8.76% (Table 1 and Fig. 1). Both parents, grasspea and pea, have interspecific and intergeneric hybridization played a significant role in integrating desirable traits of both cultivated genera in the Pappileonidae family. Using conventional methods, a number of hybrids and amphidiploids with desirable characteristics were obtained (Tandon *et al.,* 2003; Galloni *et al.,* 2007). However, there are obstacles to crossability in the majority of the other situations. Hybrid embryo culture, on the other hand, should solve these obstacles (Lord and Heslop-Harrison 1984; Rahman *et al.*, 2006). While pea is easily crossed as a female parent to grasspea, the reciprocal cross is also successful, although at a lower rate than the first form of cross (Lopez *et al.*, 2000). The advanced-generation sergeants reversion to a pure state and their hybrid viability. Fully fertile, partly fertile, and sterile Fi resulted from a cross between grasspea and pea parents. In terms of seed yield, the partly sterile and sterile crosses synthesized were inferior to both parents. As a result, there's a chance that using grasspea and pea to create a favorable relationship between nuclear genes and cytoplasm would result in beneficial recombinants that can be fixed in future generations. In the current study, a cross obtained by spraying growth regulators in combination with hybrid embryo culture allows for the incorporation of disease tolerance and other quality characteristics, as well as the removal of neurotoxin material. The combination of GA, NAA, and kinetin not only delayed pod abscission, but also facilitated the production of hybrid embryos, allowing them to be cultured. Similar spray effects have been documented in a *Vigna mungo* × *Vigna radiata* cross (Gosal and Bajaj 1983). The embryos of the two parents grew faster and showed normal development (Barpete *et al.*, 2020). The hybrid embryos excised from the young pods, on the other hand, had begun to shrivel in some cases. Furthermore, they were smaller than the same-age parental embryos. Observations of this kind have also been produced on embryos of other grasspea species also (Ehrlen and Van 2001).

Table 1: Effect of growth regulators.

Days after pollination		**0**			**3**			**6**			**9**			**12**			**15**		
Crosses		**L × P**	**P × L**	**L × L**	**L × P**	**P × L**	**L × L**	**L × P**	**P × L**	**L × L**	**L × P**	**P × L**	**L × L**	**L × P**	**P × L**	**L × L**	**L × P**	**P × L**	**L × L**
No. of pollinated buds and developing pods	**Control**	30.00	30.00	30.00	24.52	25.18	23.11	21.43	19.36	14.20	18.59	11.25	10.32	7.72	7.61	4.87	2.41	1.24	1.01
	Water application	30.00	30.00	30.00	25.14	26.54	24.36	22.05	20.19	15.81	19.26	12.42	11.45	8.33	8.44	4.45	3.65	2.63	1.27
Application of growth regulator		30.00	30.00	30.00	28.91	28.93	25.46	23.64	22.35	17.89	20.87	16.51	12.04	11.49	9.20	5.24	4.10	3.48	2.63

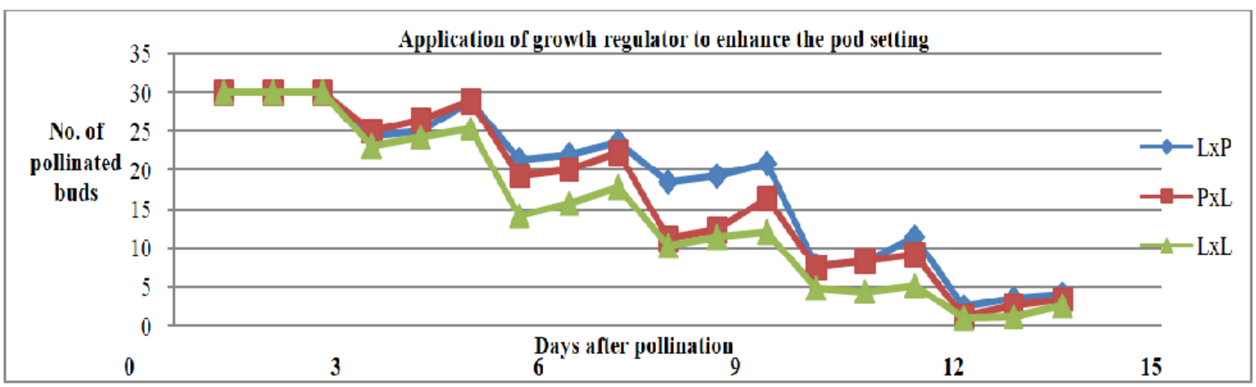

Fig. 1. To enhance the genetic pod setting by application of plant growth regulators.

A. Two types of hybridization

There are mainly two types of crosses made interspecific and intergeneric crosses. The interspecific crosses

1. Interspecific crosses: The interspecific hyridization is a cross between two different species of the same genus. Different species of grasspea (genus *Lathyrus*): *Lathyrus sativus, Lathyrus odoratus* and *Lathyrus cicera*. Out of 60 crosses only seven were set seeds while four were produce viable seeds (Table 2) and the rest three crosses produced shriveled seeds due to embryo abortion (Rahman *et al.*, 2006). The total seed obtained from 7 interspecific crosses were 29 while only 12 seeds germinated. The maximum 6 seeds set by cross *Lathyrus odoratus* × IC 142855 followed 4 seeds by cross *Lathyrus odoratus* × Ratan and *Lathyrus odoratus* × IG 64975. Out of seven crosses only four crosses produce viable and germinated seeds in which 4 from cross *Lathyrus odoratus* × IG 64975 followed by 3 in cross *Lathyrus odoratus* Ratan and *Lathyrus odoratus* × RGRLS 212-2. It shows that grasspea species are less compatible with other species.

Table 2: Interspecific crosses with total number of crosses.

Sr. No.	Cross	No. of seeds obtained	No. of plants germinated
1	*Lathyrus odoratus* × Ratan	4	3
2	*Lathyrus odoratus* × Prateek*	3	0
3	*Lathyrus odoratus* × RGRLS 212-2	3	3
4	*Lathyrus odoratus* × IC 142855*	6	0
5	*Lathyrus odoratus* × IC 142857*	3	0
6	*Lathyrus odoratus* × IG 64975	4	4
7	*Lathyrus odoratus* × IG 64869	2	2
	Total	**29**	**12**

*all seeds are shriveled and not germinated.

2. Intergeneric crosses: In intergeneric hybridization is a cross between different genera**.** In this study using different species of grasspea (*Lathyrus sativus, Lathyrus odoratus, Lathyrus cicera*) with two types of pea (*Pisum sativum*) viz., Batri and gardenpea. Out of 114 crosses 58 crosses showed successful hybridization but seven crosses viz., Mahateora × EC 243834, IC 142843 × Paras, L.O. × Paras, Indira Matar-1 × Prateek, Indira Matar-1 × RGRLS 212-2, Paras × Prateek and Shubhra × Ratan set seeds but not germinated. The total set seed obtained 299 but only 225 seeds were germinated and 74 remaining seeds are immature nonviable due to embryo abortion. The cross Ratan × Indira Matar-1 produce maximum 20 number of seeds followed by 19 seeds produced from cross Paras × Mahateora shows the compatibility of parents used in those intergeneric crosses (Verma *et al.*, 2021). For The cross Ratan × Indira Matar-1 showed maximum germination 17 seeds followed by Paras × Mahateora with 11 germinated seeds (Table 3).

Table 3: Intergeneric crosses with number of seed.

Sr. No.	Cross	No. of seeds obtained	No. of plants germinated
1.	Ratan × Indira Matar-1	20	17
2.	Ratan × Ambika	9	6
3.	RGRLS 212-2 × Iindira Matar-1	7	6
4.	Prateek × Indira Matar-1	4	4
5.	Prateek × Ambika	10	7
6.	Mahateora × Ambika	12	10
7.	Mahateora × Indira Matar-1	6	4
8.	Mahateora × EC 243834*	2	0
9.	L.O. × EC 243834	2	1
10.	L.O. × Ambika	8	6
11.	L.O. × Indira Matar-1	7	4
12.	L.O. × IC 294285	7	5
13.	L.O. × Paras*	2	0
14.	IG 64869 × Shubhra	3	2
15.	IG 64975 × Paras	3	2
16.	Mahateora × Shubhra	2	1
17.	IC 142991 × Shubhra	4	3
18.	IC 142991 × Paras	2	1
19.	IC 142991 × Indira Matar-1	2	2
20.	IC 142991 × Ambika	2	1
21.	IC 142990 × Indira Matar-1	10	9
22.	IC 142881 × Shubhra	4	3
23.	IC 142881 × Indira Matar-1	3	2
24.	IC 142881 × Paras	4	3
25.	IC 142881 × Ambika	2	2
26.	IC 142878 × Paras	4	4
27.	IC 142878 × Indira Matar	12	11
28.	IC 142857 × Paras	2	2
29.	IC 142857 × Indira Matar-1	6	4
30.	IC 142857 × Ambika	9	5
31.	IC 142855 × Indira Matar-1	10	10
32.	IC 142851 × Paras	2	2
33.	IC 142851 × Indira Matar-1	1	1
34.	IC 142843 × Shubhra	1	1
35.	IC 142843×Paras*	1	0
36.	IC 142782 × Indira Matar-1	2	2
37.	IC 142782 × Ambika	2	2
38.	Ambika × IG 64869	2	1
39.	Ambika × L.O.	2	2
40.	Ambika × Mahateora	12	10
41.	Ambika × Prateek	7	5
42.	Ambika × Ratan	7	6
43.	IC 294285 × Mahateora	3	3
44.	Indira Matar-1 × Prateek*	3	0

45.	Indira Matar-1 × Ratan	8	7
46.	Indira Matar-1 × RGRLS 212-2*	4	0
47.	Paras × IG 64869	4	3
48.	Paras × Mahateora	19	15
49.	Paras × Prateek*	3	0
50.	Shubhra × Mahateora	6	4
51.	Shubhra × Prateek	2	2
52.	Shubhra × Ratan*	2	0
53.	EC 243834 × L.O.	1	1
54.	EC 243834 × Mahateora	3	2
55.	EC 243834 × Prateek	10	9
56.	EC 356344 × Mahateora	6	5
57.	EC 356344 × Prateek	5	4
58.	IC 142855 × Prateek	1	1
Total		**299**	**225**

*all seeds are shriveled and not germinated.

B. Selection of Female parents to produce successful crosses

There were strong combiners who were able to make effective crosses and grow viable seeds. Pea popular variety Ambika as female parent showed 5 intergeneric successful hybrids with grasspea (Table 4). *Lathyrus odoratus*, as female parent 5 desirable crosses. One grasspea popular variety Mahateora and two grasspea genotypes IC 142881 and IC142991, produce 4 crosses, Four pea genotypes and out of four pea female genotypes three were popular varieties Indira Matar-1, Paras, Shubhra and one exotic genotype EC 243834 produces 3 successful intergeneric crosses, while in case of grasspea only one grasspea accession IC 142857 produces 3 successful intergeneric crosses. The result showed that Pea is better combiner as compare to the grasspea crop (Badal and Tripathi, 2021).

Table 4: Female parent ranking with successful crosses.

Sr. No.	Female parent	Number of Successful crosses with rank	Sr.No	Female parent	Number of Successful crosses with rank
1.	Ambika	5	13.	IC 142843	2
2.	*Lathyrus odoratus*	5+7 (interspecific)	14.	IC 142851	2
3.	IC 142881	4	15.	IC142855	2
4.	IC 142991	4	16.	IC 142878	2
5.	Mahateora	4	17.	Prateek	2
6.	EC 243834	3	18.	Ratan	2
7.	IC 142857	3	19.	IC 142990	1
8.	Indira Matar-1	3	20.	IC 294285	1
9.	Paras	3	21.	IG64869	1
10.	Shubhra	3	22.	IG64975	1
11.	EC 356344	2	23.	RLS 212	1
12.	IC142782	2			
		Total **58**			

FUTURE SCOPE

The main drawback of this crop is neurotoxin content ODAP content which reduces its highly manageable and valuable crop for poor farmers. It can be eliminated or reduces upto the level of <0.2% by using pure line selection, pedigree method, pre breeding distant hybridization, somaclonal variation and transformation methods so it is one of the best source of protein for people to overcome the malnutrition in developing countries.

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