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Research article

STUDY ON CROSSING ABILITY OF ANNUAL CHRYSANTHEMUM GENOTYPES

Suneetha Kattera[1*], D. M. Panchbhai[2], Mula Pratapa Reddy[3] and B. Rajasekhar Reddy[4]

[1*]PG Student, Department of Horticulture, College of Agriculture, Nagpur, India.
[2]Professor, Department of Horticulture, College of Agriculture, Nagpur, India.
[3]Research Scholar, Department of Genetics and Plant Breeding, I.Ag.Sc, BHU. Varanasi.
[4]Research Scholar, Department of Horticulture, I.Ag.Sc, BHU. Varanasi.

ABSTRACT: The experiment comprised of six genotypes *viz*., NAC-01-10, NAC-02-10, NAC-03-10, NAC-04-10, NAC-05-10, NAC-06-10 selected for petal colour and number of layers of petals to estimate pollen viability, *in-vitro* pollen germination, stigma receptivity and crossed seed set percentage to find out the crossing ability of genotypes. The present experiment was laid out at the experimental field of Horticulture Section, College of Agriculture, Nagpur, during 2010-11. Pollen viability of six genotypes of annual chrysanthemum showed a range of 69.69% to 86.66% viability, Percentage of germination on the day of anthesis ranged from 22.72% (NAC-06-10) to 66.66% (NAC-01-10). First day pollen pollinated on first day stigma showed 100% stigma receptivity in all six genotypes and the crossed seed set ranged from 61.60% (NAC-04-10 × NAC-05-10) to 92.00% (NAC-01-10 × NAC-04-10) among direct crosses whereas, the percentage ranged from 42.00% (NAC-04-10 × NAC-02-10) to 90.00% (NAC-05-10 × NAC-01-10) among the reciprocal crosses. All the six genotypes are highly suitable for their use as parents in crossing program.

Key words: Annual Chrysanthemum, pollen viability, pollen germination, stigma receptivity

INTRODUCTION

Among the flowers used for domestic market, Annual chrysanthemum (*Chrysanthemum coronarium*) is considered as one of the important commercial flowers. It belongs to family Compositae and is native to Central and South Europe. It is generally tall up to 100-120 cm with large size flower. Because of its size, shape and colour, the annual chrysanthemum is popular amongst the people. Annual chrysanthemum is one of the most important cut flower on pot plants. Breeding of new lines depends mainly on conventional crossing method. Inflorescence traits are an effective representation of ornamental merit in annual chrysanthemum. In annual chrysanthemum the flowers having small disc, more number of petals with large size, multilayered whorls of petals with multicolor has significant market value. Therefore, efforts for getting such type of genotypes are the need of the time which can be achieved by breeding / crossing technique. Success of improvement through breeding largely depends upon the choice of right parent, and breeding strategy to be adopted for the improvement. In the genus (*Dendranthema/chrysanthemum*) there are lot of variabilities which are not yet used as materials in the breeding programmes, although they have useful characters not available among the cultivars (Yang and Endo, 2005). Available genotypes also show expensive variation in flowering response which has become obstruction in cross pollination. Hence, the success rate on crossing to get new genotype is found to be less. It is therefore, necessary that study on cross ability level in annual chrysanthemum should be conducted.

MATERIAL AND METHODS

The present experiment was laid out at the experimental field of Horticulture Section, College of Agriculture, Nagpur, during September 2010 to March 2011. The experiment comprised of six genotypes *viz.*, NAC-01-10, NAC-02-10, NAC-03-10, NAC-04-10, NAC-05-10, NAC-06-10 selected for petal colour and number of layers of petals to estimate pollen viability, *in-vitro* pollen germination, stigma receptivity and crossed seed set percentage to find out the crossing ability of genotypes.

Pollen viability of genotypes (%)

The pollen viability was tested in newly opened flowers of genotypes by the method suggested by Knuth and Rose (1989). The principle of testing was to test the content of dehydrogenase (an enzyme) using one per cent solution of substrate 2,3,5-tryphenyl tetrazolium chloride (TTC) in a 5% solution. Viable pollen turns pink. Pollen from newly-opened flowers was placed on slide. A drop of tetrazolium chloride was added to the samples and a cover slip was placed over the samples. A minimum of one hundred pollen grains were counted per slide and the pollen viability (%) was worked out by using the following formula.

$$\text{Viability (\%)} = \frac{\text{Number of pollen turns to pink}}{\text{Total Number of pollen observed}} \text{ X } 100$$

Pollen fertility of genotypes (%)

To find out how long the pollen grains of genotypes remain fertile and viable from the time of anthesis, pollen germination of male parent were estimated at three intervals from the day of anthesis as per the method given by Yang and Endo (2005). The media used for germination consists of Ca $(NO_3)_2.4H_2O$(300 mg/l), $MgSO_4.7H_2O$ (200mg/l), KNO_3 (100mg/l), Sucrose (15%), Agar (1%). All the above elements were dissolved in water bath and poured into depression slides. Sprinkle the ample quantity of pollen grains on the day of anthesis, 24hrs after anthesis, and 48hrs after anthesis on the agar medium. Leave to germinate for 2hrs at 28^0C in the slides. A minimum of one hundred pollen grains was counted per slide and the pollen germination (%) was calculated by the following formula

$$\text{Pollen germination (\%)} = \frac{\text{Number of pollen germinated}}{\text{Number of pollen grains observed}} \text{ X } 100$$

Stigma receptivity of genotypes

To find out how long stigma of female parents remains receptive, pollen grains immediately after anthesis, after 24 hrs and after 48 hrs of anthesis were used to pollinate one, two and three old stigma. 3-5 stigmas were selected for each cross and observed the seed set. Stigma receptivity was calculated by using following formula

$$\text{Stigma receptivity (\%)} = \frac{\text{Number of crosses in which seed set were observed}}{\text{Total Number of crosses}} \text{ X } 100$$

Crossed seed set percentage (%)

Number of crosses done, and number of seeds obtained from each cross were noted down and crossed seed set percentage calculated by the following formula

$$\text{Crossed seed set (\%)} = \frac{\text{Number of florets in which seed set took place}}{\text{Total Number of florets crossed in each cross}} \text{ X } 100$$

RESULTS AND DISCUSSION

Pollen viability (%)

Pollen viability of six genotypes of annual chrysanthemum showed a range of 69.69% to 86.66% viability (Table.1). The genotypes NAC-03-10 (86.66%) recorded the maximum per cent of pollen viability followed by NAC-02-10 (84.31%), NAC-01-10 (82.75%), and NAC-05-10 (82.00%). However NAC-06-10 (69.69%) recorded the lowest pollen viability.

Table 1. Pollen viability (%) of parents

S. No	Parent	Viability Per cent (%)	S. No	Parent	Viability Per cent (%)
1	NAC-01-10	82.75	4	NAC-04-10	80.00
2	NAC-02-10	84.31	5	NAC-05-10	82.00
3	NAC-03-10	86.66	6	NAC-06-10	69.69

The results on pollen viability assessed by staining technique revealed that except parent NAC-06-10, all other parents exhibited 80 and above pollen viability per cent. Much difference for this character was not observed among the parents except NAC-06-10 because all the genotypes belong to same species *Chrysanthemum caronarium*. In contrary to this result Yang and Endo (2005) reported that the viability of pollen among different species were highly variable.

Pollen germination (%)

Pollen viability were also assessed by *in vitro* pollen germination test at different intervals and presented (Table 2). Pollen of genotypes was cultured in agar medium supplemented with 15 per cent sucrose, Ca $(NO_3)_2.4H_2O$ (300 mg/l), $MgSO_4.7H_2O$ (200mg/l), KNO_3 (100mg/l) at three intervals from the day of anthesis. Percentage of germination on the day of anthesis ranged from 22.72% (NAC-06-10) to 66.66 % (NAC-01-10), after 24hrs of anthesis ranged from 0.00% (NAC-06-10) to 9.23% (NAC-02-10) and after 48hrs of anthesis ranged from 0.00% (NAC-02-10, NAC-04-10, NAC-05-10, NAC-06-10) to 1.29 % (NAC-03-10). The results on pollen viability assessed by *in vitro* germination test at three different intervals indicated marked variation among the genotypes at all the three intervals. Indicating that *in vitro* germination test is more suitable than the staining technique for testing the pollen viability in annual chrysanthemum. Maximum pollen germination was observed in parent NAC-06-10 (66.66%) when cultured on the day of anthesis and 4.25% when cultured 24hrs after anthesis, and 0.98% when cultured 48hrs after anthesis. This was followed by genotype NAC-02-10 which recorded 39.13%, 9.23% and 0.00% when cultured on same day, 24hrs and 48hrs after anthesis respectively. From the result it is observed that the six genotypes used as parent can retain its viability only for one day. On the contrary Yang and Endo (2005) reported that in *D. indicum, D japonicum* viability of pollen was lost only after ten days. Similarly to this results

Table 2. Pollen germination of parents

S. No	Parent	Percentage of pollen germinated on the day of anthesis	Percentage of pollen germinated 24hrs after anthesis	Percentage of pollen germinated 48hrs after anthesis
1.	NAC-01-10	66.66	4.25	0.98
2.	NAC-02-10	39.13	9.23	0.00
3.	NAC-03-10	33.33	5.00	1.29
4.	NAC-04-10	36.84	5.12	0.00
5.	NAC-05-10	37.50	6.77	0.00
6.	NAC-06-10	22.72	0.00	0.00

HangBo *et al*. (2008) reported that pollen viability of three chrysanthemum cultivars rm1-2, rm1-3 and rm7-3 were 34.60%, 24.90%, 27.90% respectively. These reports indicate that the pollen viability per cent is highly specific to the genotypes under study.

Stigma receptivity

Stigma receptivity (%) of six annual chrysanthemum genotypes was studied by pollinating one, two and three days old pollen on one, two, three days stigma and the results are presented (Table 3). First day pollen pollinated on first day stigma showed 100% stigma receptivity in all six genotypes. First day pollen when pollinated on second day stigma, stigma receptivity ranged from 25% (NAC-06-10) to 60% (NAC-06-10). Second day pollen when pollinated on either first or second day stigma, stigma receptivity percent ranged from 20% to 50%. In all other combinations 0.00% stigma receptivity was observed.

This indicates that first day pollen is more suitable for pollination and the stigma can remain receptive only up to two days. Effective pollination and fertilization can occur when first day pollen is pollinated over first day stigma. In contrary to this result HangBo *et al*. (2008), found that receptivity period of this stigma can be maintained for 15days in some cultivars of chrysanthemum. ChunQing *et al*. (2009) reported 11.20% pollen viability in *D. levendilifolium* just before pollination, no pollen grains germinated on the stigmas during 4 hrs after pollination and only seven number of pollen grains germinated on each stigma at 8 hrs after pollination. The period of stigma receptivity was also found to be highly specific to the genotypes used for study. Similar findings were reported by YaoMei *et al*. (2007).

Table 3. Stigma receptivity (%) of annual chrysanthemum genotypes

Parent	Stigma	Pollen	Stigma receptivity (%)	Parent	Stigma	Pollen	Stigma receptivity (%)	Parent	Stigma	Pollen	Stigma receptivity (%)
NAC-01-10	1st Day	1st Day	100.00	NAC-03-10	1st Day	1st Day	100.00	NAC-05-10	1st Day	1st Day	100.00
		2nd Day	20.00			2nd Day	40.00			2nd Day	50.00
		3rd Day	0.00			3rd Day	0.00			3rd Day	0.00
	2nd Day	1st Day	60.00		2nd Day	1st Day	33.33		2nd Day	1st Day	33.33
		2nd Day	20.00			2nd Day	0.00			2nd Day	33.33
		3rd Day	0.00			3rd Day	0.00			3rd Day	0.00
	3rd Day	1st Day	0.00		3rd Day	1st Day	0.00		3rd Day	1st Day	0.00
		2nd Day	0.00			2nd Day	0.00			2nd Day	0.00
		3rd Day	0.00			3rd Day	0.00			3rd Day	0.00
NAC-02-10	1st Day	1st Day	100.00	NAC-04-10	1st Day	1st Day	100.00	NAC-06-10	1st Day	1st Day	100.00
		2nd Day	25.00			2nd Day	50.00			2nd Day	20.00
		3rd Day	0.00			3rd Day	0.00			3rd Day	0.00
	2nd Day	1st Day	33.33		2nd Day	1st Day	33.33		2nd Day	1st Day	25.00
		2nd Day	20.00			2nd Day	33.33			2nd Day	0.00
		3rd Day	0.00			3rd Day	0.00			3rd Day	0.00
	3rd Day	1st Day	0.00		3rd Day	1st Day	0.00		3rd Day	1st Day	0.00
		2nd Day	0.00			2nd Day	0.00			2nd Day	0.00
		3rd Day	0.00			3rd Day	0.00			3rd Day	0.00

Crossed seed set percentage

Percentage of crossed seed set was calculated and presented (Table 4). The crossed seed set ranged from 61.60% (NAC-04-10 × NAC-05-10) to 92.00% (NAC-01-10 × NAC-04-10) among direct crosses whereas, the percentage ranged from 42.00% (NAC-04-10 × NAC-02-10) to 90.00% (NAC-05-10 × NAC-01-10) among the reciprocal crosses. The results revealed that direct crosses resulted in more seed percentage when compare to the reciprocal crosses in most of the crosses. The seed set percentage in annual chrysanthemum on self pollination is negligible may be because of self-incompatability operating in some genotypes of annual chrysanthemum. In this study also when the plants were selfed for collecting the seeds zero per cent seed set was

Table 4. Crossed Seed Set Percentage

S. No	Crosses	Seed set (%)	S. No	Crosses	Seed set (%)
1	NAC-01-10xNAC-02-10	83.00	16	NAC-02-10x NAC-01-10	78.00
2	NAC-01-10xNAC-03-10	80.00	17	NAC-03-10x NAC-01-10	82.00
3	NAC-01-10xNAC-04-10	92.00	18	NAC-03-10x NAC-02-10	70.00
4	NAC-01-10xNAC-05-10	76.00	19	NAC-04-10x NAC-01-10	61.00
5	NAC-01-10xNAC-06-10	75.00	20	NAC-04-10x NAC-02-10	42.00
6	NAC-02-10xNAC-03-10	69.00	21	NAC-04-10x NAC-03-10	56.00
7	NAC-02-10xNAC-04-10	83.00	22	NAC-05-10x NAC-01-10	90.00
8	NAC-02-10xNAC-05-10	83.00	23	NAC-05-10x NAC-02-10	86.00
9	NAC-02-10xNAC-06-10	90.00	24	NAC-05-10x NAC-03-10	75.00
10	NAC-03-10xNAC-04-10	70.00	25	NAC-05-10xNAC-04-10	81.65
11	NAC-03-10xNAC-05-10	78.00	26	NAC-06-10xNAC-01-10	54.00
12	NAC-03-10xNAC-06-10	92.00	27	NAC-06-10xNAC-02-10	53.00
13	NAC-04-10xNAC-05-10	61.60	28	NAC-05-10xNAC-03-10	56.00
14	NAC-04-10xNAC-06-10	66.00	29	NAC-06-10xNAC-04-10	57.00
15	NAC-05-10xNAC-06-10	91.00	30	NAC-06-10 xNAC-05-10	46.00

Observed indicating that self incompatibility is operating in all the six parents used in this study. On the contrary when the same parents were involved either as male/female in the crossing program seed set ranged from 42.00% up to 92.00%. This may be because the incompatibility may be broken due to crossing the parents. The similar results were obtained by Anderson and Ascher (2000) and MyungSyun *et al*. (2007).

CONCLUSION

It can be calculated from the results on cross ability of annual chrysanthemum genotypes revealed that the six genotypes used in this study can retain the viability of the pollen for only one day and their stigma can remain receptive up to two days. All the six genotypes are highly suitable for their use as parents in crossing program.

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