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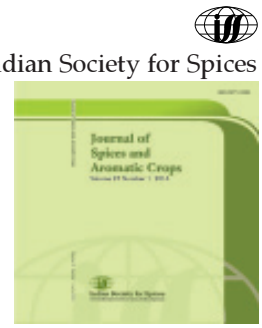

Crop improvement of coriander (*Coriandrum sativum* L. subsp. *indicum* var. *indicum*.) through crossing

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Abstract

Coriander is the most important seed spice crop in India. Selection is the most common breeding procedure used in coriander and crossing is non-existent. The present study was conducted to promote crossing as a breeding technique in coriander. It was found that coriander was protandrous and the sex ratio was 1.75 to 2.07. The stigma was receptive from the 3rd day to 6th day of anthesis indicating that artificial pollination on emasculated florets must be done repeatedly on 3rd and 4th day of anthesis. Pollen was viable for two days in the field. Fresh pollen can be stored in incubator at 25°C for three weeks with 88% retention of viability. Emasculation of florets was carried out with the help of binocular loupe. Emasculation either in the morning before anthesis or previous day evening was found to be suitable. For demonstrating the technique, four parents were reciprocally crossed. Only three crosses were successful indicating the differences in combining ability of the parents. Mean success among the crosses was 23%, fruit set among the florets varied from 9.52-83.3% depending on the cross combination.

Keywords: coriander, *Coriandrum sativum,* emasculation, crossing, hybridization

Introduction

Coriander is the most important seed spice crop in India. The full potential of the crop could not be exploited due to failure to take up systematic gene transfer, recombination, linkage-breaking, heterosis, maintenance of certain lines, maintenance of diversity in the gene pool, evaluation of parental lines and genetic analysis. Many times the problems associated with the crop was a need for crop improvement interventions through crossing. The pests and diseases such as powdery mildew, stem gall, wilt and white flies are serious in nature and need crop improvement interventions to get resistant varieties. Intensive efforts were made in erstwhile Soviet Union in the 1980s to find resistance against ramulariosis (*Ramularia coriandri*) by Romanenko and co-workers (Romanenko *et al*. 1986). They even studied the inheritance of certain traits including disease resistance (Romanenko *et al*. 1990a, 1990b). However, in

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most crop improvement attempts, selection was used in coriander and crossing was non-existent. Despite the identification of several desirable traits and resistance donors, breeding of varieties is restricted to selection alone due to absence of crossing technique. This was mainly due to herbaceous and delicate nature of the umbels, umbellets and florets thus making the crop difficult or less amenable for crossing (Diederichsen 1996). He further opined that breeding of new types in coriander need access to a wide diversity of germplasm as well as thorough understanding of floral and pollination biology of the plant. Romanenko *et al*. (1991) described the features of pollination in *C. sativum* var. *microcarpum*. Romanenko *et al*. (1992) also studied and reported the self fertility and its effects on inbreeding depression. A more elaborate and scientific taxonomy of the available diversity in the species was attempted by Diederichsen & Hammer (2003). They further described the sub species available in the Indian subcontinent and made it clear that each sub-species need to be studied independently so that more precise strategies are designed in the arena of crop improvement. Further, they opined that special type such as *C. sativum* L. subsp. *indicum* Stolet. ex Diederichsen var. *Bhutanese* Diederichsen. for high volatile oil content may be exploited further for crop improvement. López *et al*. (2008) reported that special attention should be paid to the positive correlation between essential-oil content and plant yield, because it means that it should be possible to increase the amount of seed produced and the concentration of essential oil simultaneously in a breeding program. Such improvements need a well organized and systematic program which is founded on sound knowledge of flowering and pollination biology. In this context, the present study was conducted to study the floral biology of subspecies of *C. sativum* L. subsp. *indicum* var. *indicum* Stolet. ex Diederichsen, to standardize crossing technique, demonstrate successful crosses to promote crossing as a breeding technique. The investigation was carried out during the *rabi* seasons of 2006, 2007 and 2008.

Materials and methods

The test variety was *Sudha* which belongs to *C. sativum* L. subsp. *indicum* var. *indicum* Stolet. ex Diederichsen. Floral biology was studied during three *rabi* seasons during 2006–08 using a 10x binocular loupe at Regional Agricultural Research Station, Lam, Guntur, India. The crop was raised under rainfed conditions in vertisols. A spacing of 30 × 10 cm was adopted. Plants were kept under observation for floral changes during entire crop phenology. Unopened buds were tagged and monitored for anthesis. Observations on anther dehiscence were also recorded using a stereomicroscope.

The pollen viability was studied according to the protocols proposed by Dafni & Firmage (2000). Fresh pollen was collected from recently opened anthers, from the florets of the selected plants in the field. While collecting the pollen, care was taken so that the pollen was from at least five different plants. The pollen was mixed to make a single lot. A tiny droplet of the 1% acetocarmine stain was placed on the slide. Using a teasing needle, a small amount of pollen of this lot was placed on stain and thoroughly mixed to ensure uniform penetration of the stain into the pollen. Cover slips were gently placed on to different slides for each treatment. The slides were then observed under a microscope. The stained pollen was examined under microscope and at least 500 pollen grains were counted in each sample by selecting random fields. The tests were repeated whenever there was a full staining of all the pollen grains or partial dying was observed. Pollen viability estimated thus was converted to percentage from the number of non-dyed and total pollen. Pollen viability was assessed both in the field and incubators. In the field, pollen was collected from mature anthers in a petri dish and the dishes were kept in a wooden box on a raised platform near the upper plant canopy. Pollen viability was assessed periodically at an interval of 24 h till the complete loss of viability. The viability of pollen stored in the incubator was assessed two times, i.e. 10 and 21 days after collection and was compared with that of fresh pollen.

Selfing was assessed by bagging the pre-conditioned umbels. The fully grown umbels were selected and only six hermaphrodite flowers in the outer whorl of each umbel were retained and all other flowers were clipped with a sharp scissors. Such umbels were covered with a light weight tracing paper bag and pinned at the bottom. The primary, secondary and tertiary umbels were uniformly covered to get an unbiased sample for data collection. Twenty florets in each order of umbel were used for observations. The selfing was expressed in percentage. Timeline for different stages of development of umbel from bud initiation to fruit maturity was assessed visually from a sample of 50 plants and data was recorded on a daily basis.

Stigma receptivity was assessed by deploying carefully emasculated hermaphrodite flowers of the outer whorl using a 2.5x binocular loupe. The emasculated umbels were carefully bagged. Artificial pollination was taken up using the pollen collected in the previous day and kept in an incubator. Pollination was taken up during 08:00 to 09:00 h. Sixty florets were used for each day of pollination and pollination was continued for seven days. After pollination each set of florets of that particular day was carefully bagged and assessed for fruit set after 10 days.

Data regarding variation in the order of umbels, number of umbellets within that order, floret number, and sex were recorded using 10 random plants of the test variety. Fruit set in primary umbel was assessed using five randomly selected primary umbels.

Emasculation was attempted with the help of 2.5x binocular loupe. Four genotypes (LCC-187, LCC-183, LCC184 and *Swathi*) were used and reciprocal crosses were attempted. Apart from these crosses, crosses between LCC-128 and *Swathi* were also attempted. 100 florets were used for each cross combination. Percentage fruit set was recorded after six days of pollination and at maturity.

Results and discussion

Floral biology and anthesis

Study of floral biology of the crop indicated that the crop is protandrous. The timeline for

Table 1. Timeline for different stages of development of umbel from bud initiation to fruit maturity

Day	Stage
1st day	Visible flower primordia
2nd day	Button stage
3rd day	Separation into visible umbellets
4th day	Petal development as rays in peripheral flowers
5th & 6th day	Elongation and growth of umbellets
7th day	Anthesis & pistils appear as pink
8th day	Anthesis finishes mostly (outer), pistils grow and separate, anthers shed or dry for first day opened flowers, secretions appear in flower
9th day	Pistils lose colour and well developed stigma, stigmatic secretions appear
10th day	Fruit set observed
11th day	Young fruit growth
13th day	Small sized fruits
15th day	Medium sized fruits
19th day	Well grown fruits
20th to 26th day	Fruits amass weight
27th day	Fruits show turning of colour showing the signs of maturity
33rd day	Physiologically mature and schizocarps are germinable.

flowering to fruit development is presented in Table 1. The total time taken for a flower primordial to physiologically mature fruit was ≈ 33 days. Umbel development flower primordial was in quick succession and was complete in ≈ 6 days. From the day of anthesis, fruit set was visible in ≈ 3 days and ≈ 23 days were taken for an embryo to fully mature in the schizocarp.

Stigma receptivity

The stigma was un-receptive for the first two days of anthesis and becomes receptive on the 3rd day (Fig. 1). Stigma receptivity continued from the 3rd day to the 6th day of anthesis, however, best was in the 3rd and 4th day after anthesis. In case of artificial pollination on emasculated florets, this phase of stigma receptivity demands repeat pollination on 3rd and 4th day of anthesis.

Pollen viability in field and storage

Pollen was viable for two days in the field condition and three weeks at 25°C in incubator (Table 2). From this, it can be suggested that pollen stored in incubator at 25°C may be used for artificial pollination wherever needed.

Order of umbels, sex ratio and seed set

The umbellet number gradually decreased with increasing order of umbel stature (Table 3). However, maximum number of hermaphrodite flowers was recorded in secondary umbels followed by tertiary umbels. The number of

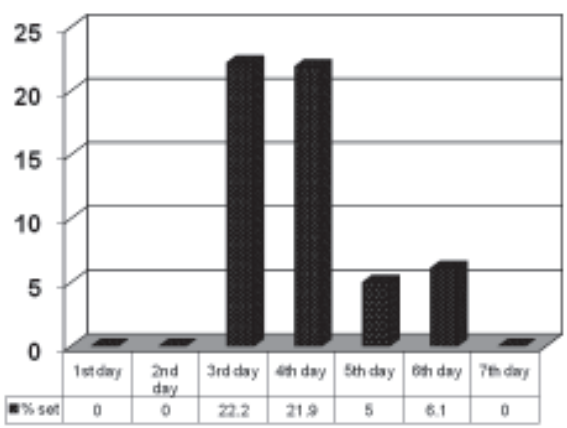

Fig. 1. Stigma receptivity of the coriander var. *Sudha*

Table 2. Pollen viability in field and storage

No. of days	% of viability	
	Field	Incubator
Fresh Pollen	98.0	99.5
1 day	90.5	99.1
2 days	82.5	99.0
3 days	0.0	99.0
10 days	-	98.7
21 days	-	88.8

Table 3. Variation among the order of umbels in umbellets, florets, sex ratio, seed set

Order of Umbel	No. of Umbellets	Florets umbellet^{-1}			Sex ratio	Seed set (%)
		Hermaphrodite	Male	Total		
Primary	7.8	4.76	9.84	14.6	2.07	51.0
Secondary	6.6	5.48	9.9	15.4	1.82	52.8
Tertiary	5.8	4.92	9.24	14.2	1.90	38.4
Quaternary	5.6	3.94	6.88	10.8	1.75	8.2

Table 4. Details of the reciprocal crosses and fruit set

Cross combination*	No. florets crossed	No. of fruits set	% set
LCC-187 X LCC-183	135	30	22.2
LCC-183 X LCC-187	60	50	83.3
LCC-184 X LCC-183	120	30	25.0
LCC-128 X *Swathi*	63	6	9.52

*Among the reciprocal crosses of 4 genotypes, only 4 cross combinations were successful

male florets was found in decreasing order with increasing order of umbel. Total florets in an umbellet were maximum in the secondary umbels. The sex ratio was found to decrease with the increasing order of the umbels.

Emasculation and crossing

Florets ready for anthesis were found most suitable for emasculation. As the florets are small and delicate in nature, binocular loupe was used for emasculation. Emasculation either in the previous day evening (15:00 to 18:00 h) or in morning (07:00 to 08:00 h) was found suitable. However, dew was an obstruction during the morning hours. The emasculated florets were bagged immediately and pollinated on the 3rd day of emasculation. Repeat pollination on 3rd and 4th day of emasculation was found better than one time pollination. Among the four parents reciprocally crossed, only three crosses were successful indicating the differences in combining ability of the selected parents (Table 4). Mean success among the crosses was 23%, fruit set among the florets crossed varied from 9.52 to 83.3% and the percentage of seed set was varied widely (10.0-80.0%). Schulenburg *et al*. (1991) reported that crosses within and between varieties of *Coriandrum sativum* and *Coriandrum macrocarpum* resulted in up to 32% seed set. Singh & Ramanujam (1972) studied the manifestation of heterosis and gene action involved in two crosses of coriander. They reported that hybrids were superior in height and yield plant^{-1}. Liehe & Rudolph (1998) reported that the parental generations as well as the F2 and F3 populations showed continuous variation in the degree of resistance to *Pseudomonas syringae* pv. *coriandricola*.

Simple crop improvement alternatives

The crop is protandrous and often cross pollinated. Natural open pollination (cross pollination) ranged from 25-70% depending on the genotype and presence of pollinators. An alternative and easy way to get hybrid population is possible. In this method, immature hermaphrodite and staminate florets are to be removed before on set of anthesis (mature florets) from the selected umbels of the female parent. These umbels are bagged and are to be pollinated on 3rd or 4th day of bagging. The fruits set contain hybrid embryos to an extent of 20-90 %. This material is used for raising F1 population along with the parents and subsequent selection is imposed to attain desired genotypes. However, in this method, there is a possibility of promoting selfed populations over generations. Identification of useful phenotypic markers to distinguish plants of selfed origin among the F1 population may be extremely useful. Use of molecular markers will help in overcoming this problem thus enabling advance of only desired populations. Apart from this, repeat spraying of Maleic Hydrazide (as chemical emasculation agent) at 125 ppm from 25 DAS onwards until the cessation of flowering has been reported to be a proven technique (Kalidasu *et al*. 2009).

The present study provided insights and solutions to several constraints encompassing crossing in coriander like period of anthesis, anther dehiscence, pollen viability, stigma receptivity, emasculation etc. Repeat pollination of florets on 3rd and 4th day of emasculation can effectively help to derive hybrid population and achieve gene transfer. Other methods such as bagging of mature florets or use of Maleic Hydrazide may also be employed as simple alternatives to cumbersome crossing.

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