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Clip-and-wash Method of Emasculation for Lettuce

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Abstract. **Complete (100%) hybridization in lettuce (*Lactuca sativa* L.) was accomplished consistently using the clip-and-wash method of emasculation. The clip-and-wash method is a combination of washing and clipping, two previously described procedures for pollen removal. The wash and the clip methods produced 98% and 95% hybridization, respectively. The method is quick and easy and eliminates inadvertent self pollination; with it one can produce the many hybrid seeds necessary for backcross and F_1 genetic analysis in lettuce.**

Genetic studies and cultivar development in lettuce are handicapped by its pollination biology and by breeders' inability to achieve 100% crosses consistently. Marker genes, such as disease resistance or morphological traits, are commonly used to distinguish hybrids and self-pollinations. The dependence on marker traits inhibits progress in a breeding program and genetic studies, since more seeds must be produced and additional plants grown to compensate for self-pollinated plants. Further, marker genes in specific crosses may be absent, difficult to identify, and/or are expressed only in mature plants, flowers, or seeds. This situation mandates lengthy waiting to determine the number of hybrids produced and increases the number of crosses needed to compensate for the inadvertent self-pollinations.

Lettuce is an obligate self-fertilizing species, which dehisces pollen before stigma emergence (Vavilov, 1935). Hence, hybridization success is determined by the effectiveness of the emasculation/depollination procedure. Oliver (1910) used a thin stream of water to remove pollen and variations of this technique are commonly used today (Ryder, 1986). Manual removal of the anthers with forceps was described by Ernst-Schwarzenbach (1932). That procedure proved to be tedious since each capitulum was an aggregate of ≈10 to 20 individual florets, each requiring separate emasculation. Pearson (1962) clipped off the corolla tips of the florets in the early morning, removing most of the anther sheath. Ryder and Johnson (1974) used an intermittent fine mist for depollination, which was a modification of the method used by Oliver (1910). Success levels for all methods described ranged from 10% to 100%. None provided 100% hybridization consistently. For example, the misting procedure described by Ryder and Johnson (1974) resulted in 57% of the crosses with 100% hybridization, 41% with 80% to 95% hybridization, and 2% with 60% hybridization. The following report describes a reliable, simple procedure of corolla clipping and pollen washing to achieve 100% hybrid seed production in lettuce.

About 30 to 60 min past sunrise, flower buds that will open that day are selected for pollination (Fig. 1A). The corolla and all floret parts contained within the bud are clipped level with the top of the involucre (Fig. 1B). Since emergence rate of the style depends on temperature and light intensity, trial and error may be needed to select the proper clipping time to avoid damaging the style and stigma. The clipped flowers are then washed with a medium fine stream of water from a hand-held misting/spray bottle. The nozzle is held ≈1 cm from the flower. Flowers are sprayed with three to four pumps of water per flower to wash away pollen, latex, and/or flower parts adhering to the bud. The flowers then are inspected about every 15 min with a hand lens for signs of style elongation. At stigma emergence, flowers are washed every 10 to 15 min for 40 to 75 min

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Table 1. Comparison of percentage of hybridization obtained using the clip and wash, clip, and wash methods of lettuce depollination.

Cross (parents)	Crosses (no.)	F_1 Seeds (no.)	Selfs (no.)	Hybrids	Hybridization (%)
			Clip and wash method		
50011 x Tall Guzmaine	21	157	0	157	100
Tall Guzmaine x South Bay	16	111	0	111	100
South Bay x Tall Guzmaine	20	191	0	191	100
Salinas x Slobolt	12	88	0	88	100
Raleigh x Baron	5	53	0	53	100
Tall Guzmaine x Slobolt	12	88	0	88	100
Total	86	688	0	688	100
			Clip method		
50011 x Tall Guzmaine	9	97	2	95	98
50011 x Ruby Red	8	68	0	68	100
Tall Guzmaine x South Bay	28	314	14	300	96
Salinas x Slobolt	17	169	7	162	96
Raleigh x Tall Guzmaine	20	187	5	182	97
South Bay x Tall Guzmaine	12	161	19	142	88
Total	94	996	47	949	95
			Wash method		
Tall Guzmaine x Ruby Red	4	17	1	16	94
South Bay x Tall Guzmaine	18	91	2	89	98
Baron x Floribibb	9	28	0	28	100
Total	31	136	3	133	98

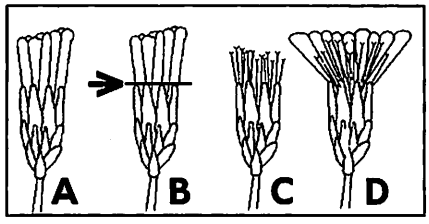

Fig. 1. Emasculation of lettuce flowers by the clip and wash procedure. (A) Capitulum at start of emasculation procedure. (B) Arrow indicates where corolla is clipped level with top of involucre. (C) Clipped and washed emasculated flower ready for hybridization. (D) Washed-only flower showing interference from corolla.

to remove all pollen grains. Important: sufficient spray pressure must be used to dislodge pollen grains, but not enough to damage the style. Normally, styles will bend during washing but should spring back when the water stream ceases. When styles are fully extended and the two lobes of the stigma are opened into a "V" shape (Fig. 1C), the washing procedure is complete. Excess water is removed from the flower by gently blowing through a straw or glass rod. It normally takes four to five washings to remove all pollen. Tags with pertinent information are secured to the flowers. Pollen is transferred to the stigmas by rotating the pollen donor flower between the thumb and index finger, allowing the styles and stigmas of both flowers to contact each other.

Eight phenotypically different parents were crossed in several combinations (Table 1). The clip and wash procedure was compared to the wash method of Oliver (1910) and the clip method of Pearson (1962). Results of the mist procedure (Ryder and Johnson, 1974) were not included due to the high rate of flower abortion associated with this technique in my study. The crosses were completed over 3 weeks and included all plants in bloom. Seeds were harvested when the involucre matured. All seeds were after-ripened at 10C and 30% relative humidity for 2 months. The seeds were individually planted into Todd planter flats, model no. 125 (Speedling, Sun City, Fla.). Hybrids were classified when 4 to 5 weeks old, i.e., when they were easily distinguished from parental types, either by leaf morphology or anthocyanin pattern.

Eighty-six flowers emasculated by the clip-and-wash method resulted in 100% hybridization of 688 seeds tested (Table 1). The clip method resulted in a 95% hybridization rate based on 94 flowers with a total of 996 seeds. The wash method resulted in a hybridization success rate of 98%.

The clip-and-wash technique permits the production of many hybrid seeds with regularity. One person may easily emasculate and cross 30 to 40 flowers in a morning. The success of this procedure may be attributed to several factors: 1) Clipping marks all the flowers for emasculation and ensures that all flowers are washed regularly. 2) Clipping removes the corolla and portions of the stamen/anther tube of each floret. When the style elongates from the tube, there is no obstruction by flower parts, and pollen removal is complete (Fig. 1 C and D). 3) Clipping shortens the stamen tube and washing may be started earlier in the day than when plants are not clipped.

The clip-and-wash procedure of emasculation described is highly efficient and dependable for obtaining lettuce crosses and will aid greatly in F_1 seed production and backcrossing procedures, especially when markers are not readily available.

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