

ETHYLENE SENSITIVITY OF CUT RACEMES OF ADVANCED BREEDING LINES OF PINK FLOWERED BLUEBONNET

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ABSTRACT

Cut racemes of Big Bend bluebonnet (*Lupinus havardii* Wats.) hold considerable promise as a new specialty cut flower crop. Over the years, as a result of our breeding and selection efforts, we have developed several lines of improved germplasm with blue, white and pink flower colors. We now have genotypes which show considerably reduced or no flower shattering. This study was conducted to evaluate the relative ethylene sensitivity of four newly developed lines (Pink Bulk, PB; Pink Light, PL; Pink Dark, PD; Pink Coral, PC) which produce different shades of pink flowers. Freshly harvested racemes were put into vases containing either water or desired concentration of 2-chloroethylphosphonic acid (CEPA) and the abscission of flowers was recorded regularly. The results indicate that the breeding lines differ widely with respect to their ethylene sensitivity. Based on the intensity of flower abscission in the presence of CEPA, the breeding line PC was found to be the most sensitive to the presence of ethylene in the vase solution, whereas the line PL appeared to be the least sensitive. A pretreatment of racemes with silverthiosulphate (STS), a known ethylene action inhibitor, prevented flower abscission even in the presence of CEPA. Earlier we reported that the sensitivity of cut racemes of *Lupinus* spp. vary widely among species. The results of this study point out that even the selections within a species may have varied sensitivity to ethylene. Recurrent selection and breeding has been quite successful in obtaining low shattering genotypes with improved vase life and longevity in Big Bend bluebonnet.

INTRODUCTION

In Texas, native lupine species (bluebonnets) have official state flower status. Big Bend Bluebonnet (*Lupinus havardii* Wats.) is native to a narrow geographical range along the Rio Grande River in southwestern Texas, and produces tall blue, fragrant racemes (2,5). Flowers are generally violet-blue, but rarely plants with white or pink flowers are also found. A research project to evaluate the cut flower potential of *L. havardii* was started in 1991. The initial focus was to improve crop uniformity and enrich our seed diversity for breeding and selection of superior genotypes. Our breeding efforts are aimed at 1) developing genotypes with novel and uniform flower colors, 2) improved vase life, 3) ethylene insensitivity or reduced ethylene production and 4) improved response to shipping. Other traits that were used for selection included low shattering and long display of flowers on the intact raceme to improve vase life (5).

The key determinants of longevity and performance of cut racemes in bluebonnet are flower abscission and senescence (4,7). Over the years, as a result of our breeding and selection efforts, we have developed several cultivars and breeding lines of improved germplasm with blue, white and pink flowers, and low ethylene sensitivity (5). This study

was conducted to evaluate the relative ethylene sensitivity of four newly developed pink flowered genotypes of Big Bend bluebonnet.

MATERIALS AND METHODS

The genetic history of the 'pink flowered' line is somewhat complicated (5). In contrast to the blue and white flowered lines, we only had one plant to begin our breeding efforts. There were four seed collected and from this we developed the pink flowered lines. Although, progress was made on postharvest longevity, color saturation, and general plant vigor, all the breeding lines originating from this single plant flowered 4-6 weeks after the blue and white flowered breeding lines. In addition, there appeared to be an increased susceptibility to disease and insect pressure in the pink flowered lines that did not occur in the blue or white flowered lines.

An exceptional year for Big Bend Bluebonnets occurred in the winter of 2000-2001. A collection trip was undertaken to determine if there were pink flowered plants in the wild population that could be used to bring new genetics into the current breeding program. Plants were found widely scattered across the Big Bend region and were pollinated and tagged for later seed collection. Seed from these rare five pink flowered plants was collected in April of 2001. In the fall of 2001 these were planted and grown in the greenhouse for evaluation. In early 2002, three plants were selected that exhibited early flowering, good flower color, and general vigor and were crossed with selected plants from an advanced pink flowered breeding line (Dark Pink 2001). Seed was collected from the crosses and planted in the fall of 2002 and designated Pink Select 2002. Plants were re-selected in early 2003 based on color, vigor, and ability of the flowers to be retained on the racemes. There were three color lines selected and pollinated separately (Dark Pink 2003, Pink Select 2003 and Coral 2003). In the fall of 2003 breeding continued on these separate lines in the same manner. From "Pink Select 2003", we obtained another distinct "Pink Light" genotype. For comparison, the original "Pink Bulk" line was also used in this study.

Plants were grown in non-shaded greenhouses at Texas A&M University Agriculture Research Center, Dallas. Cut racemes were placed in glass vases containing either 400 ml water or the test solution at $22 \pm 2^\circ\text{C}$ under cool white florescent lamps ($30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$). For evaluating the response of the cut racemes to ethylene 2-chloroethylphosphonic acid (CEPA: 100 μM , 200 μM) was added to the vase solution. Pretreatment with STS (5 mg/l) or 1-MCP (generated from 60 mg Ethylbloc™) was accomplished as described earlier (7). Observations on parameters related to postharvest display were recorded regularly.

RESULTS AND DISCUSSION

Postharvest performance of lupine racemes depends on abscission and senescence of flowers as well as on the extent of ethylene sensitivity, which varies widely both within and among species (4,7). Among the species tested, inflorescences of *L. succulentus* exhibited the highest sensitivity to CEPA in the vase solution, while *L. densiflorus* and *L. luteus* were least responsive. In *L. havardii*, among the white, blue and

pink flowered lines (Fig. 1), the “white flower” line was found to be relatively much more tolerant than the “blue” and “pink” flower lines (7). The results of the current study indicate that the newly developed “pink flower” genotypes of Big Bend bluebonnet also differ in their response to ethylene supplied as CEPA via the vase solution (Fig. 2). As compared to the original “Pink Bulk” line, the newly developed “Pink Light” line exhibited very little flower abscission and much reduced ethylene sensitivity. “Pink Dark” as well as “Pink Coral” lines also showed less flower abscission than the “Pink Bulk” line, although the flower abscission in the former two lines was much still much higher than that recorded for “Pink Light”. Thus, the order of decreasing ethylene sensitivity in pink flowered genotypes was: Pink Bulk > Pink Coral > Pink Dark > Pink Light. Among the white and blue flower lines, our current improved selection “White Select” and “Blue Select” have also been shown to exhibit very little or no flower abscission and considerably reduced sensitivity (5). It has been reported that in carnation reduced ethylene sensitivity is heritable (10). Our results with bluebonnet are in conformity with those reported for carnation.

Earlier, we reported that in a pretreatment of cut racemes of bluebonnet genotypes with either STS or 1-MCP almost completely inhibited the ethylene-induced flower abscission (7). An antagonism between CEPA and STS or 1-MCP in preventing the inhibition of ethylene-induced flower abscission was also observed in all the pink flowered genotypes tested in this investigation (data not presented). Recently, it has been reported that endogenous ethylene evokes the co-expression and accumulation of an ethylene receptor gene, ERS1, and an ethylene signaling regulator gene, CTR1, thereby speeding up flower abscission (3). STS antagonized ethylene-induced floret abscission in *Delphinium* by controlling the expression and decline of these transcripts, and thus possibly shutting off the ethylene signal transduction.

In recent years, there has been a significant outburst of new knowledge in the processes regulating abscission (6, 9). The identification and characterization of delayed abscission mutants, ethylene response mutants, MADS-box genes (e.g. shatterproof 1 and 2, Jointless, AGL-15), as well as novel genes (e.g. inflorescence deficit abscission, *ida* genes, and delayed abscission, *dab* 1-5 genes) is likely to lead to improved control of abscission and dehiscence in many plants. Additionally, several unique features of ethylene signalling and response pathways, having a multitude of inputs and outputs, have been discovered and evaluated (1, 8). New information has shed light on the components of the pathway, on the cross-talk between ethylene signaling and other hormone signaling pathways, and on the roles of transcriptional and post-transcriptional regulation of ethylene signaling (1, 8). The global analysis of ethylene-mediated changes in gene expression has uncovered hundreds of ethylene-regulated genes, providing the basis for dissecting the myriad of ethylene-mediated responses in plants (8). Genetic engineering technology could also be a very powerful tool in breeding flower crops for enhanced postharvest performance. However, in plants such as carnation and bluebonnet which have a relatively short generation time, and where several crops can be evaluated in one season, improvement by conventional breeding can also be of much practical value. Our results with bluebonnet using recurrent phenotypic selection for traits such as low flower shattering, long display life and different flower colors (Fig. 1) have clearly

demonstrated the reliability and effectiveness of selection in the development of bluebonnet cultivars with reduced ethylene sensitivity and extended vase life (5).

REFERENCES

1. Chen, Y. F, N. Etheridge and Schaller, G. E. 2005. Ethylene signal transduction. *Annals of Botany* 95: 901-915.
2. Davis, T.D., Mackay, W.A. and Sankhla, N. 2000. Distribution, biology, and potential horticultural uses of Big Bend bluebonnet (*Lupinus havardii* Wats.) – a showy winter annual from the Chihuahuan Desert. *Desert Plants* 16:3-9.
3. Kuroda, S., Hirose, Y., Shiraishi, M., Davies, E. and Abe, S. 2004. Co-expression of an ethylene receptor gene, ERS1, and ethylene signaling regulator gene, CTR1, in *Delphinium* during abscission of florets. *Plant Physiol. Biochem.* 42: 745-751.
4. Mackay, W.A., Davis, T.D., and Sankhla, N. 2001. Effect of ethephon and silver thiosulphate on postharvest characteristics of inflorescences of several lupine species. *Acta Hort.* 543:69-73.
5. Mackay, W. A., Sankhla, N., and Davis, T.D. 2005. Improvement of display life of Big Bend bluebonnet racemes by recurrent phenotypic selection. *Acta Hort.* 669: 207-211.
6. Patterson, S. E. and Bleecker, A. B. 2004. Ethylene-dependent and –independent processes associated with floral organ abscission in *Arabidopsis*. *Plant Physiol.* 134: 194-203.
7. Sankhla, N., Mackay, W.A. and Davis, T.D. 2001. Extension of vase life and prevention of ethylene-induced flower shattering in *Lupinus havardii* by 1-methylcyclopropene. *Acta Hort.* 543:75-78.
8. Stepanova, A. N. and Alonso, J. M. 2005. Ethylene signaling and response pathway: a unique signaling cascade with multitude of inputs and outputs. *Physiol. Plant.* 123: 195-206.
9. Taylor, J.E. and Whitelaw, C.A. 2001. Signals in abscission. *New Phytol.* 151:323-339.
10. Woltering, E.J. Somhorst, D. and de Beer, C.A. 1993. Roles of ethylene production and sensitivity in senescence of carnation flower (*Dianthus caryophyllus*) cultivars white sim, chinera and epomeo. *J. Plant Physiol.* 141:329-335.



Fig. 1. Pink, blue, white, and coral pink flowered breeding lines of Big Bend bluebonnet.

Fig. 2. Effect of CEPA on flower abscission in pink flowered lines after 96 hours.

