Improvement of Display Life of Big Bend Bluebonnet Racemes by Recurrent Phenotypic Selection

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Abstract

The key components affecting postharvest vase life in Big Bend bluebonnet (*Lupinus havardii* Wats.) are flower abscission and flower senescence which appear to be related to sensitivity of the germplasm to ethylene. We have used recurrent phenotypic breeding/selection techniques, including traits of low shattering and long display life of flowers on the racemes, to develop several blue, white, and pink-colored lines and cultivars of bluebonnet with reduced ethylene sensitivity and extended vase life. The relative response of the germplasm to ethylene was evaluated following treatment with 2-chloroethylphosphonic acid in the holding solution. Among genotypes tested, the advanced white-flowered line (WS02) exhibited the least sensitivity to ethylene, while the blue cultivar 'Texas Sapphire' was found to be the most sensitive. The improved breeding lines show very little or no abscission of flowers in response to ethylene. These results clearly establish the important role of selection and breeding strategies in the improvement of bluebonnet as a cut flower.

INTRODUCTION

Over the years, cut flower demand has continued to increase but domestic production has not kept pace with the growing and varied market demand. This underscores the importance of new crops in maintaining and expanding market share in the cut flower and pot plant market (Halevy, 1999). As such, new specialty cut flower crops have immense potential to expand production acreage and increase sales.

The term 'bluebonnet' is used in Texas to describe plants that most horticulturists elsewhere refer to as 'lupines' (Davis et al., 1994). Bluebonnet is considered an important symbol of Texas and all six native *Lupinus* species have official state flower status. *Lupinus havardii* Wats. (Big Bend bluebonnet) is native to a narrow geographical range along the Rio Grande River in southwestern Texas, and produces tall blue, fragrant racemes (Davis et al., 2000). 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 develop novel flower colors. Other traits that were used for selection included low shattering and long display of flowers on the intact raceme to improve vase life.

Subsequently, we discovered that the key components affecting postharvest vase life of cut racemes in *L. havardii* are flower abscission and flower senescence which appear to be related to sensitivity to ethylene (Mackay et al., 2001). Recently, inheritance and response to selection for improved vase life of flowers have been studied in gerbera (Wernett et al., 1996), Asiatic hybrid lilies (van der Meulen-Muisers et al., 1999) and carnation (Onozaki et al., 2001). This report describes some of our results relating to the development of bluebonnet cultivars and advanced breeding lines with reduced ethylene sensitivity and extended vase life using recurrent phenotypic selection.

MATERIALS AND METHODS

Plant Material

Populations of *L. havardii* occur sporadically in a narrow range in a remote area along both sides of the Rio Grande River in Texas and Mexico. In this area, several years may pass before there is sufficient rainfall to support significant populations. Flowers are generally violet-blue, but rarely white or pink flowered plants are also found. Since 1991, we have been closely monitoring the populations and have enriched our seed diversity for breeding efforts.

As described previously, we now routinely grow Big Bend bluebonnet plants in non-shaded greenhouses at Dallas, Texas. Several crops can be grown and evaluated in the greenhouse each year (Davis et al., 2000).

Crossing and Selection

Our breeding efforts are aimed at 1) developing genotypes with novel and uniform flower colors, 2) improved vaselife, 3) ethylene insensitivity or reduced ethylene production and 4) improved response to shipping.

In its native habitat, *L. havardii* requires insects, primarily honey bees and bumble bees, for pollination. However, in the greenhouse, hand pollination is readily accomplished with camel hair artist paint brushes. The plants can be easily selfed and thus rapid progress can be made in isolating recessive genes. In breeding for improved display life, we have used recurrent phenotypic selection, including traits of low shattering and long display life of flowers on intact racemes. Breeding efforts have now been carried out for more than 10 generations.

Vase Life Evaluation

Different cultivars and breeding lines of *L. havardii* were grown in the greenhouse by standard production methods. Immediately after harvest, the freshly cut racemes were brought to the laboratory for vase life evaluation.

An aqueous solution of desired concentration of ethephon (2-chloroethylphosphonic acid, CEPA) was used a source of ethylene. Cut racemes were transferred to glass vases containing either 400 ml of water or an equivalent amount of freshly prepared solution of CEPA at 22±2ºC under cool white florescent lamps (30 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). The number of abscised and senescent flowers, in control and in the presence of CEPA (100 µM) was recorded regularly, and vase life evaluated.

RESULTS

In the wild, plants with violet-blue flowers predominate. Occasionally, plants with white or pink flowers are also found in the frequency of ~1 in 10,000 for white and ~1 in 100,000 for pink. In 1992, we collected seed from pure uniform violet-blue populations, seed from four plants that were nearly white, and seed from one pink plant. When we grew the first populations in the greenhouse, we noted that there were two distinct types of plants. One type exhibited flower shattering within a few days of opening and the other type held the flowers on the raceme for a relatively long period of time. Racemes harvested from the two types exhibited the same behavior in the vase with one shattering quickly and the other exhibiting a relatively long vase life. As such, flower retention on the intact raceme became one of the characteristics that we used in our breeding effort through phenotypic recurrent selection. Within each breeding line, we routinely would select 300 seedlings based on vigor from 1000 that had been grown and then select 3-5 plants from the 300 plants that were grown from the selected seedlings for crossing or selfing, depending upon the line. Through repeated selection each year, we obtained the improved cultivars 'Texas Sapphire' and 'Texas Ice' which are violet-blue and white flowered respectively (Mackay and Davis, 1998). Subsequently, these cultivars have been further improved and refined. It is significant to note that both the 'blue flowered' and 'white flowered' breeding lines represent unbroken genetic lines derived from the initial germplasm collected in the wild in 1992. As such, 'BS02' is the latest effort in improvement of 'Texas Sapphire' and 'WS02' is the latest effort in improvement of 'Texas Ice'.

The genetic history of the 'pink flowered' line is somewhat complicated. In contrast to the blue and white flowered lines, we only had one plant to begin our breeding efforts. That plant had some undesirable traits such as lateness in flowering and susceptibility to disease and insects. We were unable to improve the time to flowering and pest resistance after several generations and decided to broaden the genetic background by obtaining new germplasm from the wild and crossing superior plants from that population with the best pink plants from the existing pink breeding line. The 'DP02' line represents our latest breeding effort in developing a superior pink flowered bluebonnet with early flowering and non-shattering characteristics.

The proportion of racemes exhibiting increased flower shattering was relatively high in the initial parental generations (data not shown). Consistent recurrent phenotypic selection over the years has resulted in production of plants that have considerably superior vase life because of very low levels of flower shattering in all three lines (Table 1). In general, the white flowered racemes were found to be less sensitive to the presence of ethylene in the vase solution, whereas the racemes of blue flowered plants were found to be more sensitive (Table 1) and exhibited higher abscission of flowers than the white flowered racemes. The results indicate that consistent recurrent phenotypic selection has also greatly improved the insensitivity of different germplasm to ethylene. Thus, our current lines appear to be superior compared to the initial germplasm in reduced flower abscission, low sensitivity to ethylene, as well as increased postharvest vaselife. The best response has been shown by the white flowered lines followed by the blue flowered lines. The latest white flowered breeding line WS02 is much superior to 'Texas Ice' and 'BS02' appears to be superior to 'Texas Sapphire'. Although improvement was noted in the pink flowered line, progress has not been as rapid and continued breeding is needed to further improve this line.

DISCUSSION

One of the most important traits affecting the value of a cut flower crop is its vase life. In the past, more effort was directed toward breeding for traits such as flower color, color uniformity, raceme length and duration of flowering or on economic traits such as productivity. However, the importance of vase life is now being increasingly recognized. Genetic improvement of vase life of flowers by crossing and selection has recently been studied in gerbera, Asiatic hybrid lilies and carnation (Wernett et al., 1996; van der Meulen-Nuisers et al., 1999; Onozaki et al., 2001). Conventional breeding techniques, in addition to providing germplasm with low abscission and increased longevity, have also been very successful in obtaining several separate color lines including dark blue, light blue, dark pink, coral pink, light pink, white, and bicolor flowers of blue/white and blue/pink in *L. havardii*. Two cultivars of *L. havardii*, 'Texas Sapphire' (blue flowers) and 'Texas Ice' (white flowers) have been released (Mackay and Davis, 1998), while these as well as several advanced lines are being further evaluated.

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 (Mackay et al., 2001). We also found that the key determinants of postharvest longevity and performance in *L. havardii* are flower abscission and flower senescence. In particular, flower abscission was found to be highly sensitive to the presence of ethylene (Sankhla et al., 1998). Application of ethephon, through the raceme axis, induced flower shattering as well as abscission of young flower buds (Sankhla et al., 2001). These results indicate that bluebonnet flowers are highly sensitive to exogenous ethylene. In general, the plants with white flowers were found to be less sensitive to the presence of ethylene compared to the blue flowered lines which were more sensitive. Recurrent phenotypic selection has resulted in reduced sensitivity of different lines. Our current improved selections, 'WS02' and 'BS02' exhibit very little or no flower abscission and show considerably reduced sensitivity to the presence of low ethephon levels in the vase. Woltering et al., (1993) reported that in carnation reduced ethylene sensitivity is heritable. Our results with bluebonnet are in agreement with those reported for carnation.

Several commercial carnation cultivars, with extended vase life, feature either a low rate of ethylene production during senescence or a reduced sensitivity to ethylene (Woltering et al., 1993; Onozaki et al., 2001). Although, we have not completed our studies on ethylene production in senescing flowers of bluebonnets lines, preliminary results indicate that plants with white flowers have low ethylene levels compared to the blue flowered lines. Similarly, although the plants were not selected specifically for ethylene insensitivity, except indirectly for low flower shattering, pronounced improvement has occurred in ethylene insensitivity as a result of repeated selection. These results suggest that as reported earlier for carnation (Onozaki et al., 2001), the vase life in bluebonnet may also be controlled by a few genes related to ethylene metabolism.

In recent years, there has been a significant outburst of new knowledge in the processes regulating abscission (Gonzales-Carranza et al., 1998; Patterson, 2001; Taylor and Whitelaw, 2001). 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, genetic engineering has significantly augmented conventional crop improvement, and holds great promise to assist plant breeders to meet the increased market demand. For instance, the vase life in carnation has been shown to be considerably extended by the introduction of an antisense ACC-oxidase gene and the *Arabidopsis* etr1-1 gene (Savin et al., 1995; Bovy et al., 1999). Thus, recombinant DNA technology could 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 an excellent alternative 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 have clearly demonstrated the reliability and effectiveness of selection in the development of bluebonnet cultivars with reduced ethylene sensitivity and extended vase life.

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Tables

Table 1. Effect of recurrent phenotypic selection on vase life parameters and ethylene sensitivity of cut bluebonnet racemes.

Breeding line/Cultivar	Percent after 96 hours				Vase life (days)
	Control		CEPA		
	*FA	*FS	*FA	*FS	
‘Texas Sapphire’	4±0.8**	12±2.0	15±3.0	28±3.6	8±0.5
BS98	17±2.5	40±2.0	94±2.0	-	7±1.0
BS01	-	-	14±2.6	6±1.5	8±1.0
BS02	-	-	-	-	9±1.0
PS98	8±1.5	21±2.2	82±1.2	21±2.5	7±0.5
PS01	5±1.5	-	20±4.0	8±2.0	8±0.8
PS02	-	-	14±2.0	2±0.6	9±0.5
‘Texas Ice’	-	8±2.4	-	10±2.0	10±2.0
WS98	-	15±1.2	28±2.0	10±1.0	9±0.5
WS02	-	-	-	-	12±1.0

*FA= Flower abscission; FS= Flower senescence

**Standard Error (±SE)

***Vase life of blue, pink and white lines in 1996-97 was 4.7-5.3 days.