

EFFECT OF NITRIC OXIDE GENERATING COMPOUNDS ON FLOWER SENESCENCE IN CUT RACEMES OF PINK FLOWERED *LUPINUS HAVARDII* WATS.

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ABSTRACT

Nitric oxide (NO*) is viewed as a diffusible multifunctional plant signal molecule. It has been shown to extend the postharvest life of a range of flowers possibly by down-regulating ethylene production. In this study, we have evaluated the effect of two nitric oxide (NO*) generating compounds (sodium nitroprusside, SNP ; N-tert-butyl- α -phenylnitron, PBN), alone and in combination with sucrose, on postharvest flower senescence of cut racemes of four advanced breeding lines (Pink Bulk, PB; Pink Light, PL; Pink Dark, PD; Pink Coral, PC) of pink flowered Big Bend bluebonnet (*Lupinus havardii* Wats.). The promotion/retardation of flower senescence depended on the concentration used and the genotype. Incorporation of sucrose in the vase solution considerably reduced the senescence of flowers, promoted growth of inflorescence axis and opening of additional flowers in PB, PL and PD. However, in PC sucrose (>30 mM) induced the wilting of the tip of the banner spot petal which eventually hastened flower senescence and flower fall. NO* donors and sucrose, when used in combination, generally exhibited a lesser degree of flower senescence as compared to those growing in solutions containing NO* donors alone. These results indicate that the beneficial or detrimental effects of NO* may depend on concentration, sensitivity of genotypes and presence or absence of sucrose in the vase solution.

INTRODUCTION

Recently, there has been an impressive upsurge in elucidating the physiological and biochemical functions of nitric oxide (NO*) in plants (2, 4, 6, 8, 13). This enigmatic, but unique diffusible multifunctional plant signal molecule, plays pivotal role in diverse plant processes including hormone modulation, programmed cell death, and wounding and defense responses. The cytotoxic or the cytoprotective roles of NO* are thought to be due to its reactivity with ROS (4, 6, 8, 13). A major breakthrough in understanding the role of NO* in plants relates to identification of multiple, enzymatic as well as non-enzymatic, NO* generating systems, and widespread production, either constitutive or induced by biotic/abiotic stresses, of NO* in plants (2, 4).

Several studies point out that there is a cross talk between NO*, ethylene, IAA, abscisic acid, GA, calcium, calmodulin, cGMP and cADPR (6, 13). NO* has also been shown to inhibit ethylene action and synthesis in plants (7), and it has been suggested that NO* acts as a natural senescence-delaying plant growth regulator primarily by down-regulating ethylene production. NO* donors have also been shown to protect a variety of cut flowers from ethylene and dramatically increase the vase life (1).

Over the years, as a result of our breeding and selection efforts, we have developed several lines of improved germplasm of *L. havardii* 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 effect of NO* donors on senescence of cut racemes of four newly developed lines of Big Bend bluebonnet (Pink Bulk, PB; Pink Light, PL; Pink Dark, PD; Pink Coral, PC) which produce different shades of pink flowers.

MATERIALS AND METHODS

Cut racemes of four advanced breeding lines of pink flowered (Pink Bulk, PB; Pink Light, PL; Pink Dark, PD; Pink Coral, PC) *L. havardii* Wats. were obtained from plants grown in a non-shaded greenhouse of trial garden at Texas A&M University, Agricultural Experiment Station, Dallas. Inflorescences were harvested in the morning and brought to the postharvest laboratory for experimentation. Sodium nitroprusside (SNP) and N-tert- α -phenylnitron (PBN) were used as the source of NO* donors. Cut inflorescences, with their freshly trimmed bases in water, were placed in glass vases containing freshly prepared solutions of the desired concentrations of the NO* donors (20 μ M, 100 μ M). Based on the results of our earlier studies, in some experiments sucrose (30 mM) was also added to the vase solution. In the vases containing sucrose, in order to reduce microbial contamination, 8-hydroxyquinoline sulphate (8-HQS) was also added regularly. The vases containing cut inflorescences in various test solutions were placed on benches in the laboratory at 22-25° C under cool white fluorescent lamps (30 μ mol·m⁻²·sec⁻¹) for 12 hours per day. The number of senescent flowers was scored daily up to 5 days, and the vase life characteristics evaluated regularly.

RESULTS AND DISCUSSION

The promotion/retardation of flower senescence by NO* donors depended on the concentration used and the genotype. The various pink flowered lines tested exhibited differential response to NO* donors (Fig.1, 2) which ranged from almost no effect or a slight inhibition to a distinct promotion of flower senescence. In genotype PD and PC a clear promotion was noticed in the senescence of flowers during postharvest vase life in SNP solution (Fig. 1). In genotypes PB and PL, depending on the concentration, the racemes either did not show any effect on flower senescence or indicated a slight inhibition. More or less, a similar response was observed in the presence of PBN (Fig. 2), although the intensity of the effect was much milder than that observed with SNP. Visible signs of flower senescence included onset of wilting and burning at the tip of the standard petal and a change in the color of banner spot. At high concentration of SNP the banner spot in PB flowers turned black, desiccated and ultimately senesced. In DP and PC dark brown patches were initiated on the banner spot during postharvest vase life. The genotype PC was found to be the most tolerant to the presence of high concentration of NO* donors. In this genotype, even at the highest concentration of NO* donors only a few flowers exhibited small brown dots on the banner spot. Earlier we observed that high concentration of SNP also brought about a change in color of banner spot from light yellow to muddy-brown/intense black in the genotype “Blue Select” (10). NO*-mediated toxicity is mainly due to its reaction with superoxide anion (O₂⁻), leading to the formation

of strong oxidant peroxynitrite, which can oxidize thiol residues to sulfenic and sulfonic acids (5). However, in soybean the HR cell death appears to be activated following interaction of NO* with H₂O₂, rather than O₂⁻ (3). Furthermore, the release of NO* into solution depends on the characteristics and concentrations of the NO* donor, the pH, temperature and concentrations of NO* target molecules (6). Thus, it becomes difficult to discriminate between the pharmacological effects and physiological relevance of the role of NO* donors and modifications induced by endogenous NO*.

Incorporation of sucrose (30 mM) in the vase solution considerably reduced the senescence of flowers (Fig. 1, 2), promoted growth of inflorescence axis and opening of additional flowers in PB, PL and PD. However, in PC sucrose (>30 mM) induced the wilting of the tip of the banner spot petal which eventually hastened flower senescence and flower fall. NO* donors and sucrose, when used in combination, generally exhibited a lesser degree of flower senescence as compared to those growing in solutions containing NO* donors alone (Fig. 1, 2).

NO* has been shown to extend the postharvest life of a range of flowers, fruits and vegetables possibly by down-regulating ethylene production (1, 7). In phlox, although SNP in the vase solution promoted the abscission of open flowers, the younger buds continued to open even in the presence of high SNP concentrations (11). On the other hand, at high SNP concentrations (> 50 μM), the leaves became either yellow, or more frequently turned progressively black and senesced (11). Inclusion of sucrose in the vase solution, or pretreatment of flower heads with either 1-MCP or STS, significantly delayed the abscission of flowers and blackening of leaves, and improved postharvest display life. Similarly, low concentrations (< 50 μM) of SNP and PBN delayed, but high concentrations (> 50 μM) promoted senescence of flowers in cut inflorescences of *L. densiflorus* (12).

Thus, it would appear that the beneficial or detrimental effects of NO* donors may depend on concentration, sensitivity of genotypes and presence or absence of sucrose and/or ethylene inhibitors in the vase solution. Also, it should be borne in mind that the multiple modes of action of NO* are suggestive of its wider modality than just on ethylene action (1). In fact, a recent analysis of NO* responsive genes based on whole genome microarray in *Arabidopsis* indicates that a wide variety of genes, including those encoding transcription factors, ABC transporters, kinases and biosynthetic genes of ethylene and jasmonic acid, are up-regulated in response to NO* treatment(9), and provide an insight into the molecular basis for the diverse functions of NO* in plants. Recent results have identified a new mechanism to modulate NO* bioactivity via non-symbiotic hemoglobin, a gene involved in arginine-dependent NO* synthesis and a novel function for NO* signaling in flowering (2). It is clear that further studies are required to dissect the exact mode of action of this multifunctional plant signal molecule.

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Fig. 1. Effect of SNP and sucrose on flower senescence.

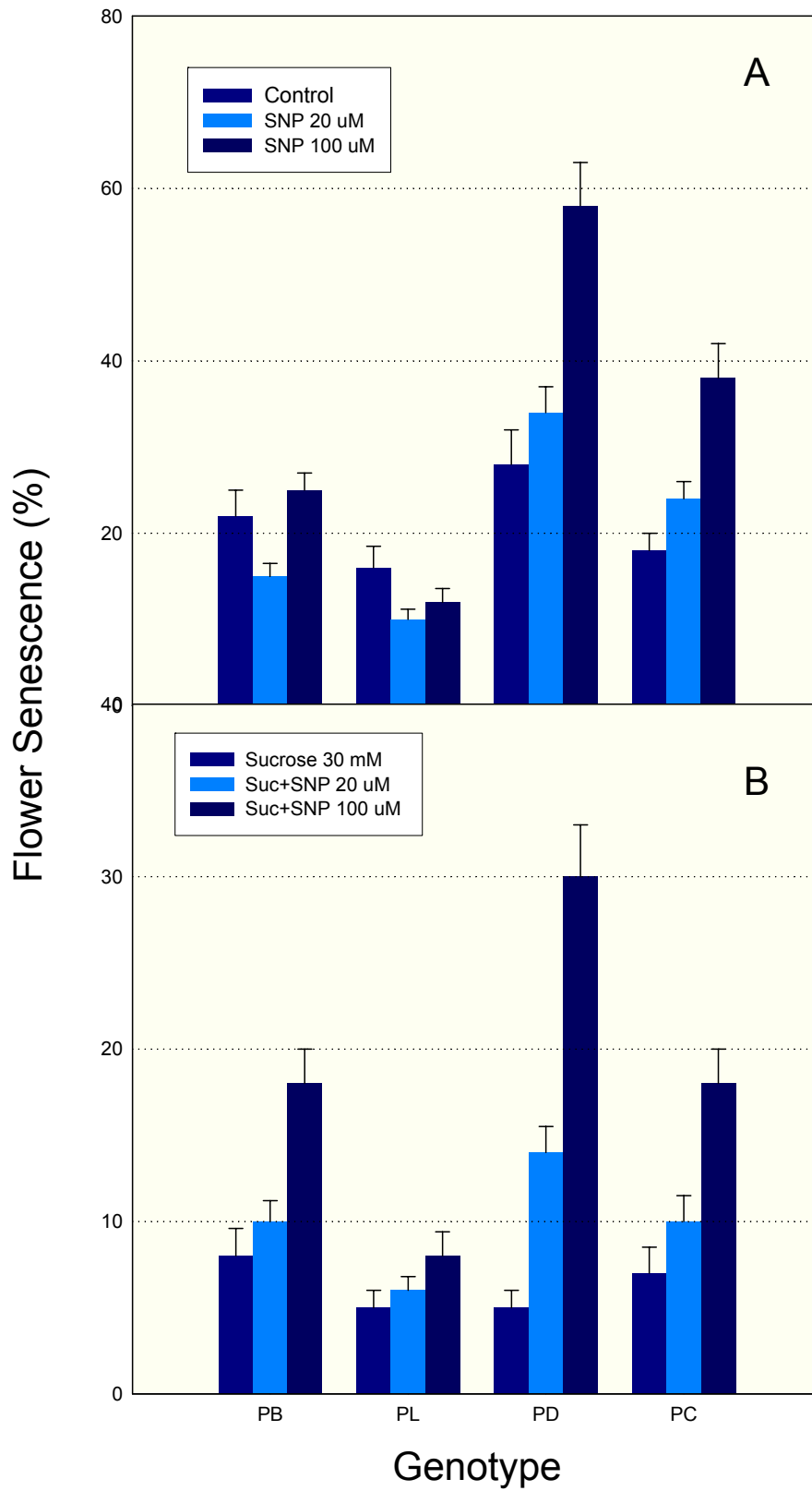


Fig. 2. Effect of PBN and sucrose on flower senescence.

