Influence of Environmental Factors on Parthenolide and Abscisic Acid in Feverfew©

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Accumulation of abscisic acid (ABA) and parthenolide (PRT) in feverfew plants exposed to different light and water conditions was investigated. The effect of light was studied by harvesting plants at different times of the day and harvesting plants exposed to either dark or light environments. PRT was found to have a maximum peak at late afternoon whereas ABA had its peak during morning hours. Light withdrawal during the afternoon resulted in reduced PRT and increased ABA. Water stress response was monitored in potted plants exposed to continuous dehydration-rehydration cycles. PRT content was higher in water-stressed plants, but only after second dehydration. In contrast, ABA was higher at first soil drying and did not increase as much in subsequent cycles. ABA inhibitors such as norflurazon and sodium bisulfite also inhibited PRT accumulation in cut feverfew flowers, indicating a connection between ABA pathway and the PRT catabolic site. Our results demonstrate that higher medicinal quality of feverfew may be obtained when harvest is during hours immediately prior to dusk and when plants have been under intermittent water stress. We provide novel evidence for understanding the physiology of the PRT accumulation in feverfew.

INTRODUCTION

Nutraceuticals are food or chemical compounds contained in food having medicinal activity. The nutraceutical industry is playing a major role in the agri-food market today. The current annual sales for herbals and vitamins alone in the U.S.A. is approximately 12 billion US dollars (Sloan, 1999). In several European countries phytotherapeutics account for 30% of all nonprescription drugs (Grimwald and Buttel, 1996). Some of the medicinal plants are also ornamental plants, a characteristic that expands their marketability. Authorities in southern U.S.A. are promoting herb production as an alternative for traditional horticultural crops. However, unlike technology for the production of ornamentals that has been developed over several decades, research targeting high quality herbs has just begun. Production of high quality herbs is a challenge for growers. Feverfew, *Tanacetum parthenium* (L.) Schultz-Bip. (Asteraceae family), a top-20 medicinal plant in sales (Bannerman, 1997), has been associated with control of migraine and of arthritis-related pain (Murphy et al., 1988). Parthenolide is considered to be the primary active compound responsible for the medicinal effects (Groenewegen et al., 1992). Herbal products are not yet regulated in the United States, but in countries with regulation feverfew quality is assessed by PRT content. The Canadian Protection Branch of Health and Welfare and the French Ministry of Health recommend 0.1% to 0.2% PRT for commercial products (Awang et al., 1991). Variability in commercial products has been shown to be significant even within the same product (Groenewegen and Heptinstall, 1986).

Medicinal compounds are part of the secondary metabolism of plants. Secondary metabolite concentration may fluctuate during the day and has been suggested to be affected by environmental stress such as insect and fungal attack, light intensity, and drought (Veit et al., 1996). Little is known about the physiology of the accumulation of PRT in feverfew. Earlier studies showed that senescent, UV-irradiated, and ethephon-sprayed plants contained lower PRT levels (Fonseca et al., 2002). These previous results with stress-producing practices suggested a relationship between the two sesquiterpenes, PRT and ABA. Water and light are two factors that could particularly influence fluctuation of these two constituents.

The influence of light prior to harvest and water stress on PRT and ABA content in feverfew was investigated to determine the connection between PRT and ABA synthesis.

MATERIALS AND METHODS

Plant Production. Feverfew seeds in 48-cell trays were germinated under an automatic mist system at Clemson University. After a month the plants were transferred to 1-gal pots. Either 6 (1 plant as experimental unit) or 15 plants (5 experimental units of three plants) were utilized for treatments in this study. All the experiments were repeated at least once and conducted in greenhouse conditions.

Sampling and HPLC Analysis of ABA and PRT. Plants were harvested and immediately transported to the laboratory inside a brown paper bag. Leaves were then quickly removed and chopped. For ABA extraction, samples of 1.25 g were placed in 25 ml 80%-methanol solution and shaken for 24 h. For PRT, leaves were dried at 50°C, ground to <500 mm particle and 150 mg were extracted with 10 ml 90% acetonitrile for 10 min. Aliquots of each extraction solution were filtered through 0.2-um membranes and injected onto a RP-HPLC system (Waters™ 1525 pump). Mobile phase for ABA quantification was 55% methanol and 45% water with 1% acetic acid at 1 ml min^{-1} for 20 min. For PRT, the mobile phase was acetonitrile and water (55 : 45, v/v) at 1.5 ml min^{-1} for 8 min. Absorbance was measured at 245 and 210 nm, respectively. Moisture was measured with a Toledo™ Moisture Analyzer and water potential with an Aqualab™ Water Activity Meter.

Effect of Light During Day of Harvest. Plants were harvested at different times of the day. In a parallel experiment, plants were covered for 4 h (3 to 7 pm) with a black plastic film to prevent any light incidence. In all cases sampling was conducted on clear sunny days.

Effect of Intermittent Water Stress. Plants were watered daily for 4 months when water was stopped until integral wilt. The plants were irrigated and then stressed to wilt again. Three cycles of dehydration and rehydration were conducted, of which results from the two first cycles are reported. Samples were taken at each stress and recovery condition and compared to unstressed controls. Moisture content ranged between 64% to 70% in wilted plants and 78% to 83% in watered plants. Water potential was –6.4 to –4.0 in wilted plants and –2.4 to –1.1 in turgid plants.

Effect of ABA inhibitors. Flowers, with pedicels and leaves, were cut from 4- to 5-month-old plants and placed into 980-ml jars containing different solutions. Flowers were utilized since this organ has the highest accumulation of PRT (Hendriks et al., 1997). Treatments were 100 mM sodium bisulfite, 10 mM norflurazon, and distilled water. After 36 h in the solutions all flowers were transferred into jars containing 5% sucrose solution. After 12 h, the flowers were removed from solutions and remained dehydrating for exactly 6 h.

RESULTS AND DISCUSSION

Accumulation of PRT and ABA clearly fluctuates during the day. PRT increased with time during the light period with a peak before dusk. During night hours PRT declined. ABA started to increase at night and continued through the morning when it reached maximum levels, then decreased in the afternoon (Fig. 1). The influence of light during the day of harvest was examined when light was removed from the plants' environment. PRT decreased and ABA increased in plants exposed to dark for 4 h prior to sampling (Fig. 2). The significance of photosyntetically active radiation (PAR) for enhancement of other secondary metabolites (e.g., indigo in wood) has also been reported (Stoker et al., 1998). Interestingly, the accumulation of PRT and ABA seems to coincide with the diurnal fluctuation of the enzymes zeaxanthin epoxidase (ZEP) and 9-cis-epoxycarotenoid dioxygenase (NCED). Both enzymes appeared to be clue in the synthesis of ABA prior the formation of ×anthosal (Seo and Koshiba, 2002). Highest activity of ZEP was observed in the middle of the light period, whereas the peak of NCED was found to be at the end of the light period (Audran et al., 1998; Thompson et al., 2000).

Initial water stress resulted in plants with higher ABA and lower PRT content. However, in subsequent dehydration and rehydration cycles, ABA was lower and PRT increased (Fig. 3). This was consistent in two separate experiments, and in a trial conducted in a commercial field in which PRT was ca. 30% higher in plants without irrigation (data not shown). The low response of the plant to accumulate "stress" ABA under returning dehydrations has been reported previously (Liu and Dickmann, 1992; Zhang and Tardieu, 1996) and has been suggested to be due to the consumption of the dedicated pool of carotenoids available for the formation of "stress" ABA (Milborrow, 2001).

Flowers submitted to norflurazon and sodium bisulfite reduced ABA accumulation as expected but it also significantly reduced PRT content in flowers (Fig. 4). The observed PRT decline in flowers under water (control) is probably due to maturity effect as we have found that postflowered plants have reduced PRT in leaves (data not shown). Moreover, we have observed that petals are high in PRT, but this could change in late stages of senescence (data not shown).

Three possible pathways have been suggested for the synthesis of ABA (Millborrow, 2001; Seo and Koshiba, 2002). Norflurazon inhibits the enzymes involved in the dehydrogenation reactions leading from the phytoene to α-carotene (Bartels and Watson, 1978), a precursor of lycopene and α-carotene. Our results indicate that PRT formation follows carotenoids synthesis, which takes place before any of the proposed ABA pathways. Moreover, sodium bisulfite is expected to quench

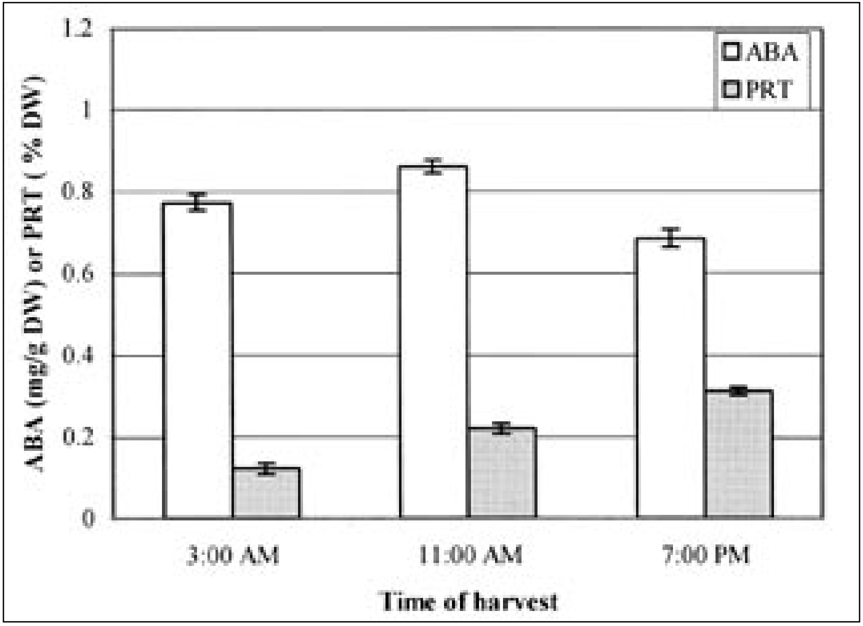

Figure 1. Influence of time of harvest on PRT and ABA levels in feverfew. Bars indicate ±SE.

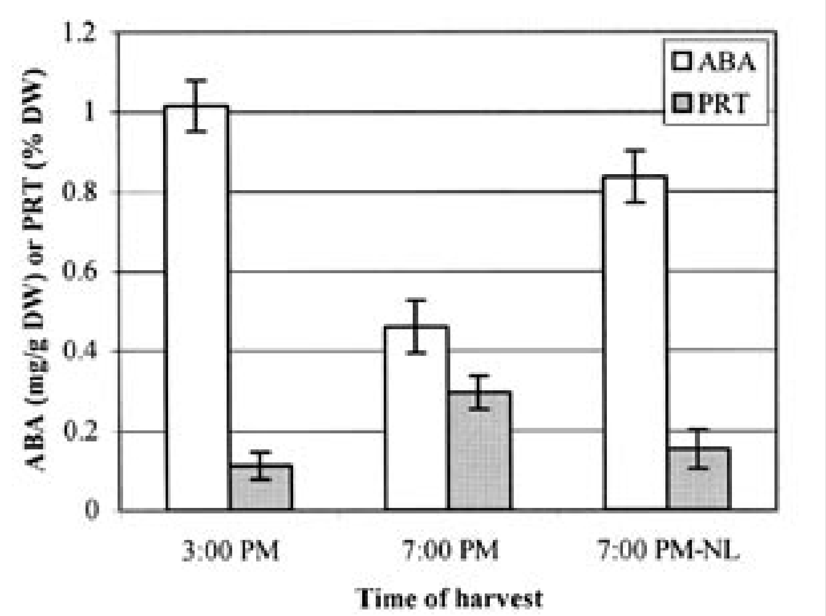

Figure 2. Influence of light period interruption on PRT and ABA levels in feverfew. NL indicate exclusion of light from 3 to 7 PM prior to sampling. Bars indicate ±SE.

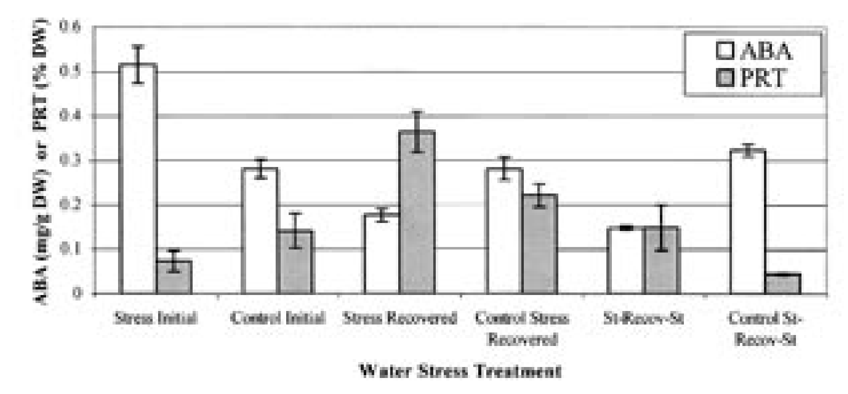

Figure 3. Influence of water stress-recovery cycles on the PRT and ABA levels in feverfew. Bars indicate ±SE.

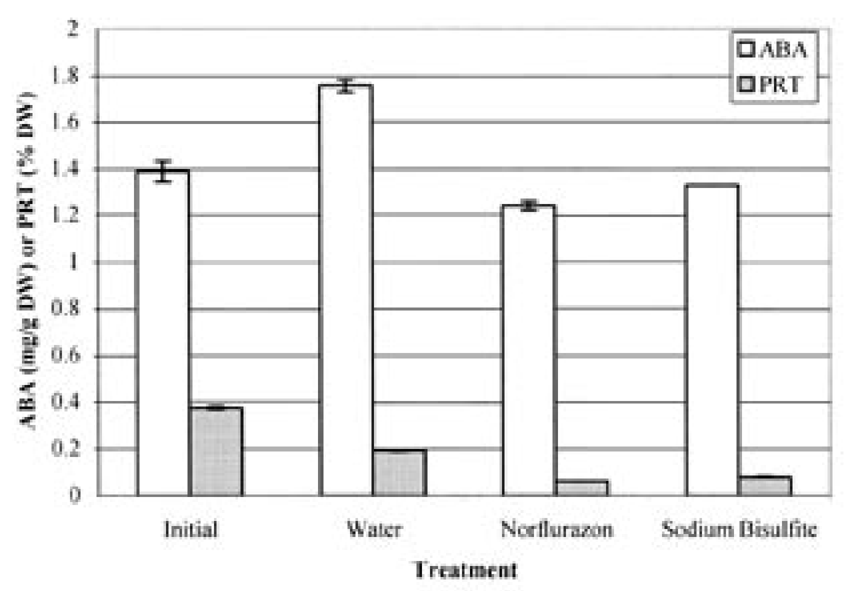

Figure 4. Influence of ABA inhibitors on PRT levels in feverfew. Bars indicate ±SE.

ABA synthesis by forming a complex with ABA-aldehyde (Millborrow, 2001). This compound is believed by many to be the most possible immediate precursor of ABA, however, not all agree (Cowan, 2000). The observed PRT inhibition with sodium bisulfite is intriguing and should be taken with care since high levels of salt could affect other metabolic processes (Salisbury and Ross, 1992). Work is in progress studying the effect of other ABA inhibitors such as naproxen and sodium tungstate in both vegetative and floral tissue. Parthenolide formation could derive from a ramification of the ABA pathway or rather from catabolism of ABA.

In this study results reveal an insight into the synthesis of PRT in feverfew. A connection between the ABA pathway and the production of PRT seems reasonable at this time; however, correlation between each compound accumulation was not obtained. It appears that different environmental factors have different effects on PRT and ABA. The most important point is that evidence suggest that feverfew should be harvested in late afternoon for highest medicinal quality. In addition, our results support that elevated PRT in feverfew may be obtained with production systems that include intermittent irrigation-desiccation cycles prior to harvest.

LITERATURE CITED

Audran, C. 1998. Expression studies of the zeaxanthin epoxidase gene in Nicotiana. Plumbaginifolia. Plant Physiol. 118:1021-1028.

Awang, D.V.C., B.A. Dawson, and **D.G. Kindack.** 1991. Parthenolide content of feverfew (*Tanacetum parthenium*) assessed by HPLC and H-NMR spectroscopy. J. Nat. Prod. 54:1516-1521.

Bannerman, J.E. 1997. Echinacea #1 in natural food trade. HerbalGram. 41:53

Bartels, P.G. and **C.W. Watson**. 1978. Inhibition of carotenoid synthesis by fluridone and norflurazon. Weed Sci. 26:198-203.

Cowan, A.K. 2000. Is abscisic aldehyde really the immediate precursor to stress-induced ABA? Trends Plant Sci. 5:191-192.

Cutlan, A.R., L. E. Bonilla, J.E. Simon, and **J. E. Erwin**. 2000. Intra-specific variability of feverfew: Correlations between parthenolide, morphological traits and seed origin. Plant Med. 66:612-617.

Fonseca, J.M., J.W. Rushing, N.C. Rajapakse, R.L. Thomas, and **M.B. Riley**. 2002. Medicinal quality of greenhouse-grown feverfew as influenced by preharvest conditions. Proc. South. Nurs. Assoc. 47:84-87.

Grimwald, J. and **K. Buttel**. 1996. The European phytotherapeutics market, figures, trends, analyses. Drugs made in Germany. 39:6-11.

Groenewegen, W.A and **S. Heptinstall**. 1992. Progress in the medicinal chemistry of the herb, feverfew. Prog. Med. Chem. 29:217-238

Hendriks, H. Y. Anderson-Wildeboer, G. Engels, R. Bos, and **H.J. Woerdenbag**. 1997. The content of parthenolide and its yield per plant during the growth of *Tanacetum parthenium*. Planta Medica 63:356-359.

Liu, Z and **D.I. Dickmann**. 1992. Abscisic acid accumulation in leaves of two contrasting hybrid poplar clones affected by nitrogen fertilization plus cyclic flooding and soil drying. Tree Physiol. 11:109-122.

Lois, R. 1994. Accumulation of UV-absorbing flavonoids induced by UV-B radiation in *Arabidopsis thaliana* L. Planta 194:498-5030.

Millborrow, B.V. 2001. The pathway of biosynthesis of abscisic acid in vascular plants: A review of the present state of knowledge of ABA biosynthesis. J. Exp. Bot. 52:1145-1164

Murphy, J.J., Heptinsall, S., and **J.R.A Mitchell**. 1988. Randomized double-blind, placebo-controlled trial of feverfew in migraine prevention. The Lancet. 11:189-192

Salisbury, F.B. and **C.W. Ross**. 1992. Plant Physiology. Wadsworth, Inc. Belmont, California.

Seo, M. and **T. Koshiba**. 2002. Complex regulation of ABA biosynthesis in plants. TRENDS Plant Sci. 7:41-48.

Sloan A.E. 1999. Top Ten Trends to watch and work on for the millennium. Food Technol. 53:40-60

Stoker, K.G., D.T. Cooke, and **D.J. Hill**. 1998. Influence of light on natural indigo production from woad (*Isatis tinctoria*). J. Plant. Growth Regul. 25:181-185.

Thompson, A. J. et al. 2000. Abscisic acid biosynthesis in tomato: Regulation of zeaxanthin epoxidase and 9-cis-epoxycarotenoid dioxygenase m RNAs by light/dark cycles, water stress and abscisic acid. Plant Mol. Biol. 42:833-845.

Veit, M., W. Bilger, T. Mulhlbauer, W. Brummet, and **K. Winter**. 1996. Diurnal changes in flavonoids. J. Plant. Physiol. 148:478-482.

Zhang, J. and **F. Tardieu**. 1996. Relative contribution of apices and mature tissues to ABA synthesis in droughted maize root systems. Plant Cell Physiol. 37:598-605.

Feeding Preferences of *Agraulis vanillae* (Gulf Fritillary) for *Pentas lanceolata* cultivars©

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A study conducted in 2002 determined feeding preferences of *Agraulis vanillae* L. (gulf fritillary butterfly) for *Pentas lanceolata* (Forssk.) Deflers cultivars. *P. lanceolata* (pentas) are herbaceous annuals in the majority of the United States and commonly recommended nectar sources for attracting butterflies. Through hybridization *P. lanceolata* are produced in a wide range of flower colors and growth habits. One cultivar, 'Lilac Mist', attracted a greater number of total inflorescence visits, feeding visits, total plant trips, and longer visit duration by the gulf fritillary butterfly than the remaining five cultivars evaluated. Differences in morphology and color characteristics were found among cultivars. However, these differences did not correlate with the observed feeding preference.

INTRODUCTION

Butterfly gardening has become a popular niche in horticulture. Fueled by the popularity and increasing number of butterfly centers at public botanical gardens across the nation, the public has embraced the idea of attracting butterflies to their home landscapes. The horticultural industry has responded by marketing and producing plants that serve as nectar sources to attract butterflies to tap into this growing market. Considerable knowledge exists regarding the species of plants that are important nectar sources. However, over the years, extensive successful hybridization of these plants by the horticultural industry has produced many cultivars of the same species with a wide range of flower colors and growth habits. These additional cultivars meet consumers aesthetic needs, but their effectiveness for attracting butterflies is not known. Research has shown butterflies can be highly selective in their