Effect of putrescine on MDA, proline and sugars in *Matricaria chamomilla*

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**ABSTRACT:** In this study investigated the effect of putrescine sprayed on Chamomile (*Matricaria chamomilla* L.) and the changed of MDA, proline and sugars were examined. The results show that put reduced MDA to compare control. But the proline content increased under put treatments. Also solution sugars content accumulate while solution sugars in 0.5 mM Put is more than the 1mM.

**Keywords:** Chamomile, *Matricaria chamomilla* L., Proline, putrescine.

**Abbreviations:** MDA, malondialdehyde; PA, polyamine; PAs, polyamines; Put, putrescine; Spd, spermidine; Spm, spermine

**INTRODUCTION**

Chamomile (*Matricaria chamomilla* L.) is one of the important medicinal herb native to world. This plant can be found in North Africa, Asia, North and South America, Australia. Chamomile has been used in herbal remedies for thousands of years, known in ancient Egypt, Greece, and Rome (Issac, 1989). As a drug, it finds use in flatulence, colic, hysteria, and intermittent fever (Tyihak et al., 1962). The flowers of *M. chamomilla* contain the blue essential oil from 0.2 to 1.9%, (Mann and Staba, 2002; Bradley, 1992) which finds a variety of uses. Chamomile is used mainly as an antiinflammatory and antiseptic, also antispasmodic and mildly sudorific (Mericli, 1990). Chamomile tea eye washing can induce allergic conjunctivitis. Pollen of *M. chamomilla* contained in these infusions are the allergens responsible for these reactions (Subiza et al., 1990).

Polyamines can be considered as one of the oldest group of substances known in biochemistry (Galston 1991). Nowadays, Put and Spd are believed to be ubiquitous in all living cells. Many results support the contention that polyamines are essential for life. Thus, chemically or genetically induced depletion of Put and/or Spd levels is lethal in yeast, protists and plants (Urano et al. 2005). Organisms deficient in Spm are viable, but show different degrees of dysfunction. This indicates that Spm, albeit not essential, must also play very important roles in growth and development (Yamaguchi et al. 2007; Minguet et al. 2008).

A perusal literature indicates that no reports are available about the effects of putrescine, on *Matricaria chamomilla*. In this study investigate that changes of some physiological parameters (MDA, Proline & Solution sugars) in *Matricaria chamomilla* that treatments with putrescine.

**MATERIALS AND METHODS**

**Plant growth and treatments**

In order to investigate the effect of Put on chamomile, treatments arranged in a randomized complete block design with 3 replications. Seeds germinated in greenhouse for two weeks. Then seedlings transferred to farm. After one month the seedlings sprayed with Put (G1: 0 mM Put, G2: 0.5 mM put and G3: 1mM Put) six times at two weeks. Finally the necessary samples of leaves were taken for each experiment.
MDA content

Malondialdehyde (MDA) contents were measured using a thiobarbituric acid reaction (Heath and Packer, 1968). About 0.5 to 1.0 g of tissue was homogenized in 5 ml of 5% (w/v) trichloroacetic acid and the homogenate was centrifuged at 12000g for 15 min at room temperature. The supernatant was mixed with an equal volume of thiobarbituric acid (0.5% in 20% [w/v] trichloroacetic acid) and the mixture was boiled for 25 min at 100 °C, followed by centrifugation for 5 min at 7500g to clarify the solution. Absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the A600. MDA contents were calculated using an extinction coefficient of 155 M⁻¹ cm⁻¹.

Other aldehyde content (Propanol, butanol, Hexanol, etc.)

Other aldehyde content were measured according to the method of Miers et al., 1992. About 0.2 to 0.5 g of tissue was homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid and the homogenate was centrifuged at 10000g for 5 min. The 1mL supernatant was mixed with 4mL of thiobarbituric acid (0.5% in 20% [w/v] trichloroacetic acid) and the mixture was boiled for 30 min at 95 °C, followed by centrifugation for 10 min at 10000g to clarify the solution. Absorbance of the supernatant was measured at 455 nm and corrected for non-specific turbidity by subtracting the A600. Other aldehyde content were calculated using an extinction coefficient of 0.457 × 10⁵ M⁻¹ cm⁻¹.

Estimation of proline

Proline was estimated according to the method of Bates et al. (1973). Five gram of leaves was homogenized with 3% sulfosalicylic acid and the content was centrifuged at 10,000 g. A volume of 2 ml of glacial acetic acid and 2 ml of acid ninhydrin was added to 2 ml of tissue homogenate and incubated for 1 h in boiling water bath followed by cooling in ice bath. About 4 ml of toluene was then added and mixed vigorously. The chromophore containing toluene was aspirated from aqueous phase and the absorbance was measured at 575 nm.

Solution sugars content

Solution sugars content was measured based on acidic hydrolyzed of sugars with sulfuric acid and production of colored furfural complex according to the method of Roberts et al., 1959. Leaf tissues (500 mg) were homogenized in ice bath with 5 ml water. The homogenate was centrifuged at 12000 g for 15 min. One ml of phenol 5% and 3 ml of sulfuric acid were mixture with supernatant. The reaction was carried out for 1 h in darkness and absorbance was measured at 485 nm. The amount of sugars content was calculated using a standard curve prepared with known concentrations of sugar.

Statistical Analysis

All experiments were recorded in triplicates. Data were subjected to analysis of variance and means and standard errors calculated. The means were compared using Duncan's test at p ≤ 0.05. SPSS (version 16) was used for this purpose.

RESULTS AND DISCUSSION

MDA content were showed that Put treatments decreased MDA in chamomile (Figure: 1). Data of MDA show that 0.5 mM Put decreased MDA content more than 1mM Put. Compare different value between all groups is significant (ps0.05).

Measuring of other aldehydes in seedlings represent decreased under Put treatments to compare control (Figure: 2). The other aldehydes content in G3 is lower than G2. Different value between G2 and G3 are not significant (ps0.05).
proline content increased under Put treatments significantly. Different value between G2 and G3 is significant also the proline content in G3 is higher than the G2 (Figure: 3). Content of solution sugar indicate is not significant different in G1 and G3 groups. Solution sugar content of G2 display accumulation dramatically (Figure: 4). Different value between G1 & G3 is not significant while the G1<G3.
In the early stages of polyamine research, Richards and Coleman (1952) observed the presence of a predominant unknown ninhydrin positive spot that accumulated in barley plants exposed to potassium starvation. After isolation and crystallization, this compound was identified as Put.

Further work in different plant species has shown that polyamine accumulation occurs in response to several adverse environmental conditions, including salinity, drought, chilling, heat, hypoxia, ozone, UV-B and UV-C, heavy metal toxicity, mechanical wounding and herbicide treatment (for review see Bouchereau et al. 1999; Alcázar et al. 2006b; Groppa and Benavides 2008). However, the physiological significance of these responses remained unclear, and it had to be evaluated whether elevated polyamine levels were a result of stress-induced injury or a protective response to abiotic stress.

Classical approaches, using exogenous polyamine application and/or inhibitors of enzymes involved in polyamine biosynthesis, pointed to a possible role of these compounds in plant adaptation/defense to several environmental stresses (for a review, Bouchereau et al. 1999; Alcázar et al. 2006b; Groppa and Benavides 2008). More recent studies using either transgenic overexpression or loss-of-function mutants support this protective role of PAs in plant response to abiotic stress (Alcázar et al. 2006b; Kusano et al. 2008; Gill and Tuteja 2010).

CONCLUSION

PAs are natural materials in plants and their changes with growth condition. If the effects of PAs in plants will be understood then the growth and developments in plants will be cleared. Polyamines such as Put show that has effect on MDA, proline and other properties on chamomile.

REFERENCES