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ROLE OF CERTAIN OXIDISING ENZYMES IN THE FLOWER DEVELOPMENT AND EXPANSION IN *SPATHODEA COMPANULATA* BEAUV.

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Changes in the activities of catalase (E.C. 1.11. 1.6), peroxidse (E.C. 1.11.1.7) and polyphenol oxidase PPO (E.C. 1.14.18.1) was studied during the growth and development of the flower in *Spathodea companulata.* Catalase activity was constant initially showing an increase at stage 3 and declined during stage 4. In mature open flower, high catalase activity is associated with calyx and gynoecium. Peroxidase activity was high initially and then declined which again increased at maturity. Polyphenol oxidase (PPO) showed similar trend. The growth phase of flower development was characterised by low peroxidase activity. At maturity high peroxidase activity was associated with corolla and gynoecium. PPO activity was initially high and decreased with the progression of flower development. At maturity high PPO activity was associated with corolla. The bud-liquid showed activity of all the enzymes at stages 2 and 3, which decreased from stage 2 to 3. The bud-liquid seems to be obligatory for flower development in *S. companulata.*

Keywords: Catalase; Flower development; Peroxidase; Polyphenol Oxidase; Spathodea companulata.

Introduction

Flower development in general has been recently reviewed[1,2] and a hormonal regulation model of floral expansion has been suggested[3]. The enzymatic oxidation of various carbon compounds by molecular oxygen without the involvement of electron carriers is referred to as direct oxidation and the enzymes involved are known as direct oxidases, which include catalase, peroxidase and polyphenol oxidase. The activity of these enzymes has been studied during growth, development and mostly senescence[4-7]. However, not much is known about the involvement of these enzymes during growth, development and opening of a flower. In the present investigation, apart from the various other aspects studied,[8-10] changes in the activities of catalase, peroxidase and polyphenol oxidase during the development and opening of flower in *Spathodea companulata* Beauv. *in vivo* are reported. *S. companulata* is horticulturally important plant and is unique as the flowers accumulate a sort of bud-liquid in inflated calyx during the early stages. This liquid is reabsorbed during the growth and opening of flowers.

Materials and Methods

Fresh flowers and flower buds were collected from *S.companulata* trees growing in the botanical gardens of Osmania University. Various stages of flower development were sampled randomly. The developmental process of flowers was divided into four stages based on the morphological differentiation and maturation as described earlier[8-10]. The floral parts of the stage 4 (open flower) were analysed separately and designated as 4K- Calyx, 4C- Corolla, 4A-Androecium and 4G- Gynoecium. The bud-liquid was

also analysed and designated as2L and 3L for stages 2 and 3 flower buds respectively.

The extraction and assay of catalase (E.C. 1.11.1.6), peroxidase (E.C.1.11.1.7) and polyphenol oxidase (PPO) (E.C. 1.14.18.1) was carriedout according to chance and Maehly[11]. The activity of catalase was expressed as enzyme units calculated.[11] Peroxidase and PPO activities were expressed as absorbancy units A 420. The activities of these enzymes were expressed per g.f.w. and per ml of bud-liquid. For the different floral parts of stage 4 open flower, the activities of the enzymes were expressed in two ways(a) as per floral part per flower and (b) as per g.f.w. inorder to obtain a better understanding of their status in different floral parts. All the results were statistically analysed and S.D. values calculated.

Results and Discussion

Amongst the important and thoroughly worked out enzymes involved in direct oxidation during growth, development and senescence are catalase, peroxidase and polyphenol oxidase (PPO). These enzymes are involved in scavenging of hydrogen peroxide (H_2O_2) formed during various metabolic processes involved in growth, development and senescence.

The H_2O_2 formed from free radicals is lethal to the plants and is split into H_2O_2 and O_2 by catalase, peroxidase and also by PPO in the presence of phenolic substrates.

Catalase activity remained more or less constant initially but increased during the phase of active cell division reaching its peak during early phase of cell elongation at stage 3 (Fig. 1). Cell elongation is usually associated with loosening of cell wall accompanied by certain hydrolytic actions. These may result in a higher production of H_2O_2 and a probable high catalase activity. The catalase activity declined sharply in the mature open flower. Thus the catalase activity appears to be positively correlated with both cell division and more so with cell elongation. High catalase activity was observed in the bud-liquid at stage 2 and declined at stage 3, whereas the buds showed reverse trend in catalase activity (Fig. 1). Of all the enzymes studied involved in the direct oxidation, catalase activity is highest during the active phase of cell division and elongation suggesting a more closer association of catalase with the developmental process of *Spathodea* flower. At maturity (Stage 4), high catalase activity was associated with calyx (4K) and corolla (4C) on floral part basis, whereas on g.f.w. basis 4C reflected less catalase activity. 4K showed high catalase activity because calyx is photosynthesising organ and would have photorespiration which in turn result in the formation of H_2O_2 and probably high catalase activity[4,7] (Fig. 2 and Fig. 2 inset). Camp[4] reported high catalase activity in male than in the female plants which was more pronounced in floral structures. Increase in catalase activity during panicle initiation in rice has been shown[7] and is correlated with the formation of H_2O_2. However, not much is known about catalase activity during flower development.

Peroxidase destroys H_2O_2 in the presence of a wide range of hydrogen donors and is relatively unspecific for hydrogen donors. Initially peroxidase activity was higher but declined during the later phase of cell division at stage 2 and remained

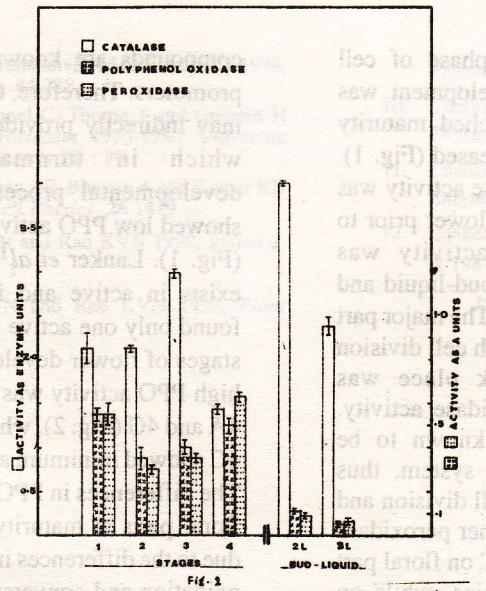
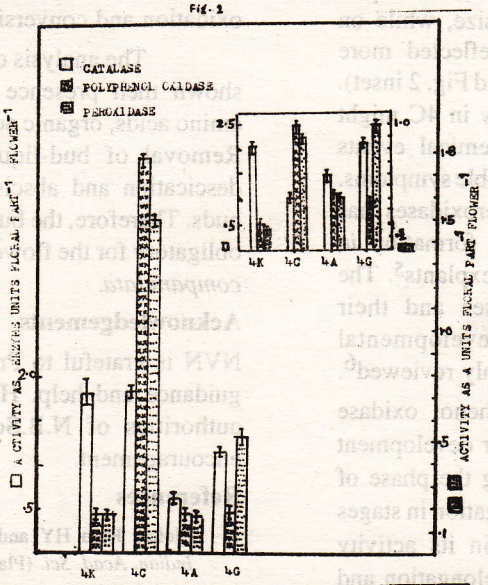


Fig. 1



Changes in the activities of catalase, peroxidase and polyphenol oxidase (PPO) enzymes :

Fig. 1. during growth and development of *S. companulata* flower.

Fig. 2. in the different floral parts of stage 4 open flower expressed on floral part per flower basis. 4K-Calyx, 4 C-Corolla, 4 G-Gynoecium and 4A-Androecium.

Fig. 2. inset-in the different floral parts of stage 4 open flower expressed on g.f.w. basis.

Vertical bars represent S.D. values.

constant during the early phase of cell enlargement. Once the development was completed and flowers reached maturity and opened, its activity increased (Fig. 1). Ultimately highest peroxidase activity was associated with the mature flower prior to pollination. Peroxidase activity was comparitively lower in the bud-liquid and slightly decreased at stage3. The major part of the growth phase, in which cell division and cell elongation took place was characterised by low peroxidase activity. The peroxidases are also known to be invovled in IAA oxidation system, thus suggesting auxin induced cell division and elongation. At maturity, higher peroxidase activity is associated with 4C on floral part basis due to its dominant size, while on g.f.w. basis 4G and 4A reflected more peroxidase activity (Fig. 2 and Fig. 2 inset). The high peroxidase activity in 4C might indicate the start of biochemical events prior to the appearance of visible symptoms. An increase in cathodic peroxidases has been shown during flower formation *in vitro* in tobacco epidermal explants[5]. The involvement of peroxidases and their isoenzymes in various developmental processes has been thoroughly reviewed[6].

Changes in polyphenol oxidase (PPO) activity during flower development revealed low activity during the phase of active cell division and elongation in stages 2 and 3 and an increase in its activity towards the compeltion of elongation and floral maturity. However,it has a high initial activity at stage 1 (Fig. 1). The lower PPO activity and high phenolic content during the phase of active growth and development (Charyulu,communicated) indicate a positive correlation. Certain phenolic compounds are known to act as growth promoters. Therefore, the low PPO activity may indirectly provide these compounds, which in turnmay regulate the developmental process. The bud-liquid showed low PPO activity at stages 2 and 3 (Fig. 1). Lanker *et al*[12] showed that PPO exists in active and inactive forms, and found only one active form during various stages of flower development. At maturity high PPO activity was associated with 4C, 4A and 4G (Fig. 2), whereas on g.f.w. basic 4C showed minimum activity (Fig. 2 inset). The differences in PPO activity in different floral parts at maturity could probably be due to the differences in the rate of phenolic oxidation and conversion.

The analysis of the bud-liquid has shown theh presence of phenolic acids, amino acids, organic acids auxins etc.[8-10]. Removal of bud-liquid resulted in the descication and abscission of the flower buds. Therefore, the bud-liquid seems to be obligatory for the flower development in *S. companulata.*

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