Changes in the activity of nitrogen utilizing enzymes during development of malformed and normal panicles of mango (*Mangifera indica* L.)

V.K. Singh, J.P. Saini and A.K. Misra

Central Institute for Subtropical Horticulture, Rehmankhera, PO. Kakori, Lucknow – 227 107, India.
E-mail: vks_lko@yahoo.com.in

Abstract

Nitrogen utilizing enzymes *viz.*, nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) were assayed during the developmental stages of healthy and malformed mango panicles in cultivars Amrapali and Dashehari. The study was conducted on healthy and malformed panicles at (I) fully developed apical bud (II) flower bud at inception (III) fully grown panicles prior to full bloom and (IV) fully developed panicle at full bloom stage. Nitrate reductase activity was significantly reduced in normal panicles from stage I to IV in both the cultivars, however, non significant changes occurred at different developmental stages of malformed panicle in cultivar Amrapali. In general, the activity of GS and GOGAT followed the same trend. Contrary to the activity of NR, GS and GOGAT, a sharp increase in GDH activity was noticed in malformed panicle at early stage of panicle development and its maximum activity was noticed at stage II in Amrapali (77.0 mM α - Ketoglutarate g^{-1} FW h^{-1}) and Dashehari (95.10 mM α- Ketoglutarate g^{-1} FW h^{-1}), and declined gradually during stage III and IV. The higher activity of GDH in malformed panicle indicated the shift of primary route of ammonia assimilation (GS/GOGAT) at early stage of panicle development. Level of total nitrogen, protein and nitrate content declined with the age of healthy panicle in both the cultivars, however, their level remained higher at later stage of malformed panicle development.

Key words: Mango, malformation, nitrogen utilizing enzymes, panicle, floral

Introduction

Etiology of mango malformation has been controversial and the malady has variously been considered to be acarological (Attiah, 1955), fungal (Summanwar *et al.*, 1966) and physiological in nature (Sattar, 1946, Tripathi, 1955, Majumder *et al.*, 1970, Bastawros, 1986). The disease causes considerable damage to the mango production in some parts of India (Kumar and Chakrabarti, 1997) and elsewhere (Schlosser, 1971).

Various physiological and biochemical parameters, associated with floral malformation, have been described by many workers (Pal *et al.*, 1983, Singh and Dhillon, 1988, 1993). Nitrogen metabolism is one of the key systems in growth and development of plant but despite of it's importance, current knowledge about the role of nitrogen metabolic enzymes in mango malformation is fragmentary. Therefore, the present investigation was undertaken to study the principal enzymes of nitrogen metabolism at different stages of healthy and malformed panicles of mango cultivars Amrapali and Dashehari.

Materials and methods

The present study was carried out at Central Institute for Subtropical Horticulture, Lucknow during the years 1995-1997, on uniform plants of cultivars Amrapali and Dashehari (10 years old). The shoots bearing healthy and malformed buds at different stages viz., (I) fully developed apical bud (II) flower bud at inception of panicle growth (III) fully grown panicles prior to full bloom and (IV) fully developed panicles at full bloom, were selected for studies.

In vivo nitrate reductase activity was measured in healthy and malformed tissues by the method of Srivastava (1975) in which the assay mixture in a final volume of 5.0 ml contained 80 mM potassium phosphate buffer (pH 7.4), 50 mM n-propanol and 10 mM KNO_3. The enzyme activity was measured in terms of nitrite released into the medium by adding 1 ml sulphanilamide (0.1% in 1N HCl) and 1 ml of 0.02% N-1-napthylethyline diamine dihydrochloride (NED).

Glutamine synthetase (GS) activity was estimated in the cell free extract of healthy and malformed tissue, by the method of Sddique *et al.* (1982). Samples were crushed in chilled mortar and pestle with cold Tris-HCl buffer 50 mM (pH 7.8) which contained 1.0 mM mercaptoethanol, 2.0 mM EDTA and 2% PVP. The homogenate was filtered through cheese cloth and centrifuged at 20,000 X g for 20 min at 4°C. The supernatant was used as the source of enzyme. The assay mixture in a final volume of 3.0 ml contained : 40 mM imidazole-HCl buffer (pH 7.6), 0.25 mM ATP, 12 mM sodium arsenate, 1.5 mM $MnCl_2$, 30 mM glutamine and 25 mM hydroxylamine (pH 7.0). The mixture was incubated for 30 min at 30°C. The reaction in a sample (1.5 ml) of incubated mixture was terminated by 0.5 ml $FeCl_3$ which then produced a brown compound (λ max$_{540}$) γ – glutamyl hydroxamate (GHA). The GS activity in terms of Mn^{2+} dependent γ – glutamyl – transferase reaction was determined using GHA as standard and expressed as m mol GHA h^{-1} g^{-1} fr wt.

Glutamate synthase (GOGAT) activity was measured as per Singh *et al.* (1983). Samples were crushed in chilled mortar and pestle with 3 ml of cold 0.2 M Tris-HCl buffer (pH 8.0)

containing 0.1% (v/v) mercaptoethanol and 2 mM EDTA. After raising the volume to 5.0 ml the filtrate was centrifuged for 25 min at 4°C. The supernatant thus obtained was used as the enzyme source. The assay mixture in a final volume of 1.5 ml contained : 1.0 ml Tris-HCl buffer (0.1 M pH 8.0), 0.2 ml α – Ketoglutaric acid (2.0 mM), 0.1 ml NADH (0.1 mM), and 0.2 ml glutamine (10.0 mM). The estimation is based on the disappearance of α – Ketoglutarate (α – KG) detected in 0.5 ml of the incubated assay mixture with a reagent that contained : 0.5 ml 2, 4-dinitro phenyl hydrazine (1 mg ml^{-1} in 2 M HCl), 0.5 ml Na_2CO_3 (10%), 0.5 ml benzyl alcohol and 2.5 ml of 1.0 M NaOH that develops a yellow colour with λ max at 420 nm.

Glutamate dehydrogenase (GDH) activity was measured by the method of Mathur *et al.* (1983) in which the assay mixture in a total volume of 1.5 ml which contained : 1.0 ml Tris-HCl buffer (0.1 M, pH 8.0), 0.2 ml α – Ketoglutaric (0.2M), 0.1 ml NADH (0.005 M), and 0.2 ml NH_4Cl (1.5M). The disappearance of α – Ketoglutaric acid in the assay mixture was tested as in the case of GOGAT.
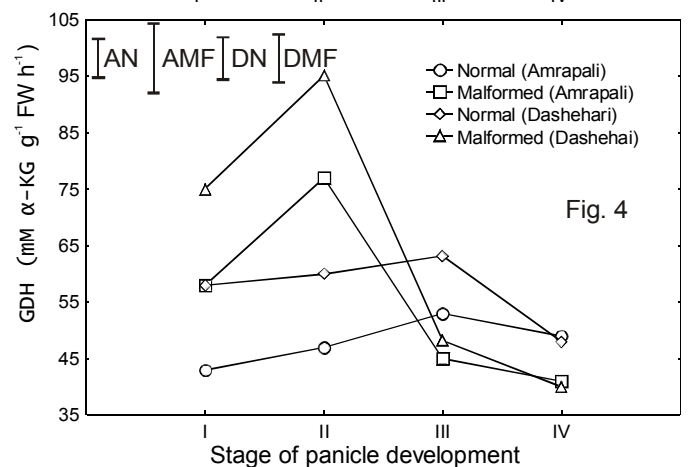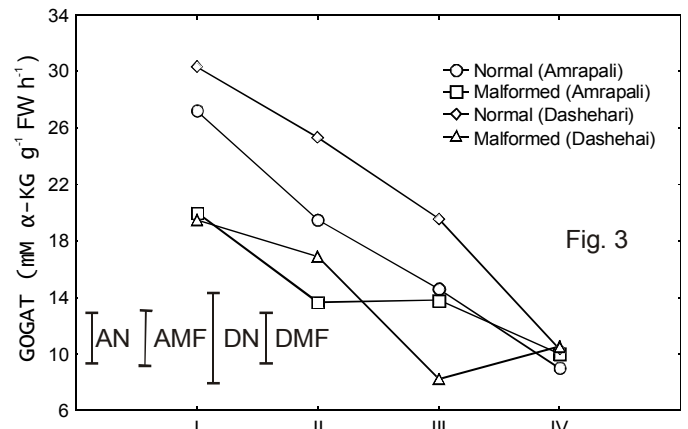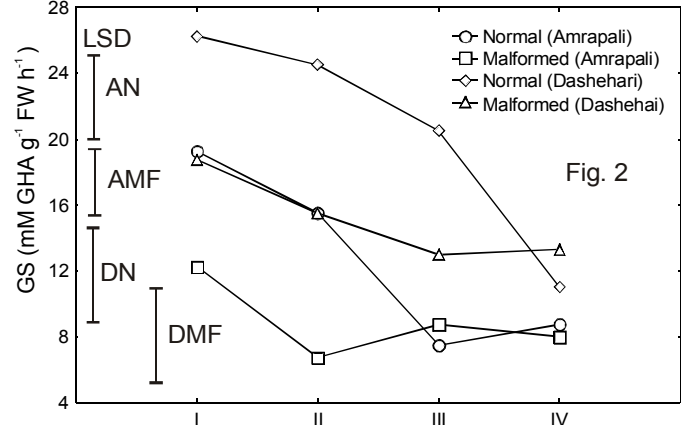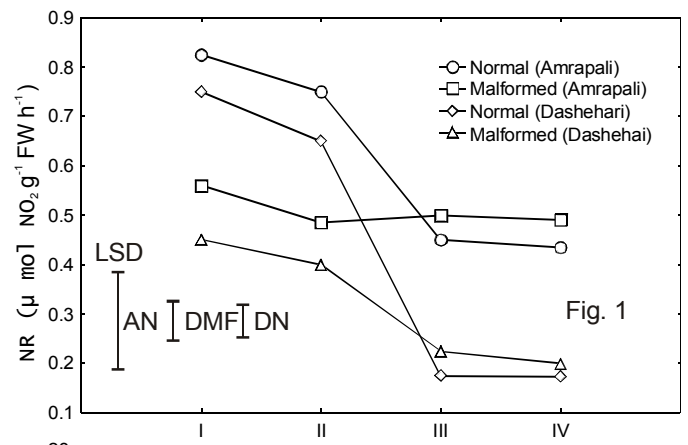
Nitrate and protein content was estimated by following the method described by Singh (1988) and Lowry *et al.* (1951), respectively and nitrogen by microjedhal method.
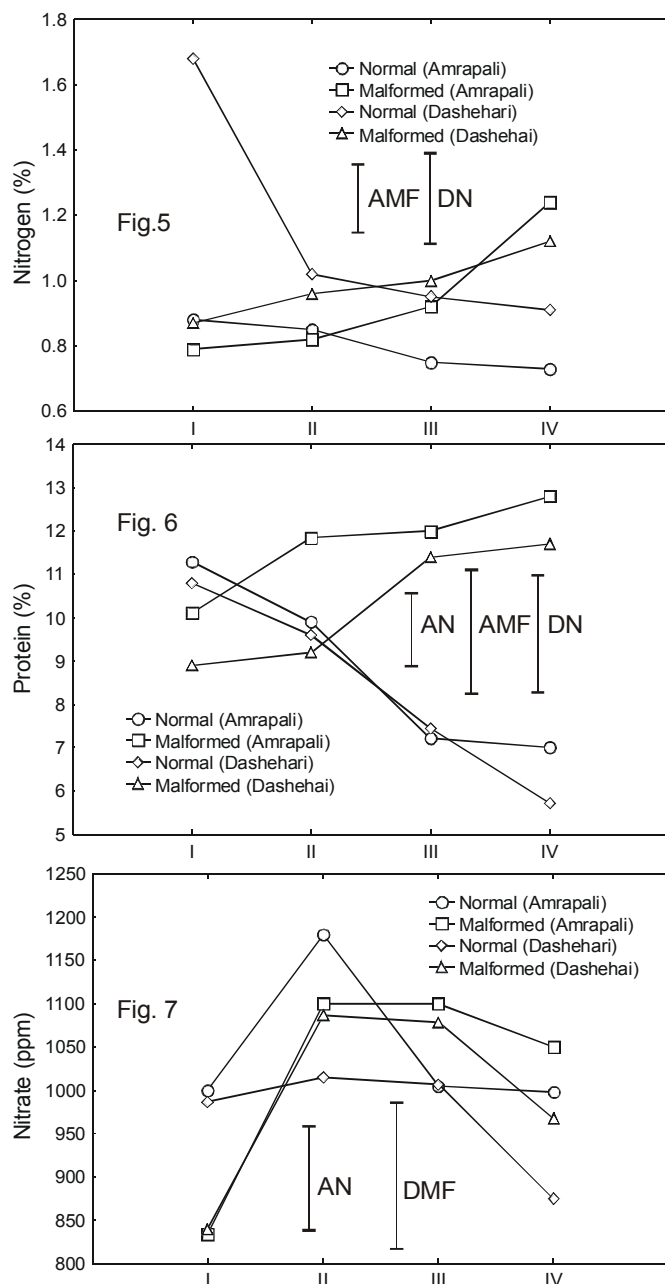
Results and discussion

It is clear from the Fig. 1 that the level of nitrate reductase activity was significantly reduced from stage I to IV in normal panicles of both the cultivars. Its maximum activity was observed at stage I and II when flower bud development took place. Activity declined sharply in stage III when panicle attained full growth. Decline was more in the case of Dashehari, while Amrapali showed comparatively less reduction. Very little change in it's activity was recorded in stage IV. On the other hand, NRA exhibited non significant differences at different stages of malformed Amrapali panicles.

The higher accumulation of nitrate (NO_3) in healthy panicles than malformed ones was observed during stage I but in stage II to IV, malformed panicles exhibited more accumulation (Fig. 5). Increase in NO_3 accumulation was observed upto the stage II and later it decreased in stage III and IV of panicle growth in both healthy and malformed tissues. Non significant difference in NO_3^- level at different stages of malformed panicle was recorded in case of Amrapali whereas in Dashehari it was highly significant. The variation in NRA in Amrapali and Dashehari might be due to different type of localization or compartmentation of nitrate within the cells of these two cultivars. Similar information has also been reported in other crops (Beevers and Hageman, 1969).

NRA is a substrate (NO_3) inducible enzyme (Haynes, 1986), but in the present investigation, the higher amount of nitrate in the malformed tissues as compared to healthy at II to IV stages of the flowering in both the cultivars do not seem to induce NRA, significantly. Therefore, it is postulated that the intracellular mobilization of NO_3 to the site of utilization of NR activity may be inhibited in malformed tissues. Restricted mobilization of metabolites has also been reported by other workers (Pandey *et al.*, 1977).

Changes in the activitiy of nitrogen utilizing enzymes: NR (**Fig. 1**), GS (**Fig. 2**), GOGAT (**Fig. 3**) and GDH (**Fig. 4**) in panicles at its differnet growth stages in Amrapali and Dasehari mango. Vertical bars show LSD (*p*=0.05) values for enzyme activity at different stages.

Changes in the levels of nitrogen (**Fig. 5**), protein (**Fig. 6**) and nitrate (**Fig. 7**) in panicles at its differnet growth stages in Amrapali and Dashehari mango. Vertical bars show LSD (*p*=0.05) at different stages.

In general, the GS activity declined from stage I to other subsequent stages. It was significantly reduced in normal as well as in malformed panicle from stage I to IV, in both the cultivars (Fig. 2). Contrary to Dashehari, non significant difference was noticed in GS activity of normal malformed panicles of Amrapali. Maximum reduction in GS activity in malformed panicle of Amrapali and Dashehari was obtained at stage II. At stage II and III of healthy panicles of cultivar Amrapali and at stage III and IV of Dashehari it showed maximum reduction. Similar trend was observed in case of GOGAT activity, however, pattern of GOGAT activity in both the cultivars was somewhat different than the former two enzymes. Contrary to the NRA and GS activity, GOGAT activity

was significantly reduced in malformed panicle when compared with normal ones in both the cultivars.

Unlike NR, GS and GOGAT, the GDH activity was higher in malformed panicles as compared to healthy ones in stage I which increased significantly in stage II and again declined gradually in stage III. While in case of healthy panicles, it's activity was low throughout the panicle development (Fig. 4). It is interesting to note that, maximum GDH activity in Amrapali (77.0 mM α - KG g^{-1} FW h^{-1}) and Dashehari (95.10 mM α - KG g^{-1} FW h^{-1}) in malformed panicles was observed at stage II, when flower bud development takes place. Low activity of enzymes at later stages of flowering and initial stage of fruit development, which may be due to lower rate of anabolic activity of panicle and limited substrate availability. Variation in the activity of enzymes in Amrapali and Dashehari at various stages of panicle development may be due to their genetic differences.

Assimilation of ammonia/amino acid takes place by two pathways *viz*., (i) GS/GOGAT and GDH pathway. This is inevitably dependent on the concentration and availability of NH$_4^+$ (Angelabs and Kliewer, 1992). However, GS/GOGAT is considered as the primary route of ammonia assimilation in higher plants (Miflean and Lea, 1977). In the present study, higher values of GS and GOGAT in healthy panicles over malformed ones clearly lends supports to the view that GS/ GOGAT pathway works more efficiently in healthy panicles than malformed ones. Similar information on GS / GOGAT pathway has also been reported in other crops(Oaks and Hirel, 1985).

On the other side, two possibilities can be considered to explain the noticeable increase in GDH activity of malformed panicles. Firstly, more production of ammonia in the malformed panicle (Singh and Dhillon, 1988), which may enhance GDH activity (Miflein and Lea, 1977, Singh *et al.*, 1986). It is thus, possible that increase in GDH activity in malformed tissues is a consequence of the increased concentration of NH$_4$. Secondly, increased GDH activity is possibly a mechanism of detoxification of ammonia.

It is noticeable that stage I and II were more important in differentiating the activity of nitrogen utilizing enzymes rather than stage III and IV. These stages are critical as the fast metabolic changes take place during this period, which ultimately effect the panicle. It is therefore derived that, in depth, study in stage I and II may give more valuable information in the understanding of enzyme activity in flower bud development and floral malformation. The sharp increase in the GDH level at early stage of flower bud initiation could be used as an indication of floral malformation. Further, the use of specific inhibitor of GDH activity particularly for its action in the development of mango malformation consequently its control needs thorough investigation. The data pertaining to protein content (Fig. 6) clearly revealed that its level decline with the age of healthy panicles in both the cultivars, but decline was not associated in normal panicle of Amrapali as its growth progressed. On the other hand, the nitrogen as well as protein content in Dashehari did not vary significantly in malformed panicle at different stages of development. The accumulation of metabolites may be due to low catabolic activity of malformed tissue, which could cause the excessive growth of malformed

panicles. Similar results have been reported by earlier worker (Singh and Dhillon, 1988, 1993). It is concluded from this study that like other metabolic imbalance, the disturbed amino acid assimilation pathway may also be one of the stemming factors for creating the disorder of malformation.

Acknowledgement

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