

## ***IN VIVO* AND *IN VITRO* ESTIMATION OF SOME SELECTIVE METABOLITES OF *MESUA FERREA* (LEAF CALLUS)**

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Medicinal plants are potential source of raw materials used for manufacture of medicines and structural products. Metabolites are directly or indirectly involved in the growth and development of plants. In the present investigation, the *in vivo* leaf (without PGRs) and the callus of leaf explant of *Mesua ferrea* developed on various concentrations of PGRs were evaluated for their biochemical estimation of selective metabolites viz., protein, proline, phenolic, nitrogen and total sugar using various methods. Results showed maximum content of protein in the callus raised on MS +4.0 mg/l 2,4-D (5.82±0.121 mg/gdw), proline in MS+1.0 mg/l 2,4-D+1.0 mg/l BAP (1.06±0.167 mg/gdw). Phenolic in MS+4.0 mg/l 2,4-D (101±30.024 mg/gdw), nitrogen in MS+3.0 mg/l 2,4-D (0.675±0.015 mg/gdw) and total sugar in MS+1.0 mg/l 2,4-D (2.08±0.087 mg/gdw). It further indicates that callus developed from the leaf explant of *Mesua ferrea* is a rich source of metabolites and can be used in pharmaceutical industries.

**Key words:** *Mesua ferrea*, nitrogen, protein, proline, phenolic, , total sugar.

Beneficial and medicinal properties of plants have been known since long and have been used as sources of food, fodder, oil, medicine, fuel, wood, fibers and timber. Medicinal plants have been the subject of man's curiosity since time immemorial (Constable 1990). The primary benefits of using plant-derived medicines are that, they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Bandow *et al.* 2003). The study of plants continues principally for the discovery of novel metabolites. *Mesua ferrea* is an important member of family Clusiaceae (Guttiferae). It is commonly known as Nagkeser. It is the national tree of Srilanka and also found in tropical India. It is highly valuable plant for its high structural and medicinal value. The wood of this plant is very heavy, hard and strong, weight is about 72 lbs per cubic foot and density is 1.12kg/m<sup>3</sup>. Colour of the wood is dark deep red. It is used for making rail road ties and structural timber (Kakrani 1984). Its resin is slightly toxic, but many parts of this plant have medicinal properties. It enhances the complexion. It leads to fragility and transparency to the skin. It is useful in conditions like asthma, leprosy, cough, fever, vomiting, and impotency. The flowers are acrid, anodyne, digestive, constipating and stomachic. Dried flowers are used for bleeding piles, menorrhagea,

menorrhagea, excessive thirst and sweating. Oil from the seeds is used for sores, scabies, wounds and rheumatism (Bhide *et al.* 1977, Jain and Jain 1973). It also has the antibacterial, antifungal, anthelmintic properties and shows excellent anti-inflammatory and styptic activity.

Metabolites directly involved in growth and development of plants, viz. amino acids, nucleotides, carbohydrates and lipids have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation, and are also used in industries as raw materials and food additives (Akindele *et al.* 2007). In the present study, plant *Mesua ferrea* of family Clusiaceae (Guttiferae), is evaluated for its biochemical estimation of some selective metabolites viz. protein, phenolics, proline, nitrogen, and total sugar, as so far, no such report on this important plant is available.

### **MATERIAL AND METHODS**

For the present study *Mesua ferrea* has been used as experimental material. The explants were procured from the recently introduced nursery named Tau Devilal Medicinal Garden, Tajewala (Haryana) in the C.C.S. University campus, Meerut. The leaves of the plant are selected as explant for the induction of callus. The explants were surface sterilized

subsequently with running tap water, Tween - 20 (detergent) and finally with 0.1% HgCl<sub>2</sub> for 5 minutes under laminar air flow. The explants were then thoroughly rinsed with doubled distilled sterilized water 2-3 times and the leaves were excised and directly inoculated on MS medium (Murashige and Skoog 1962) under aseptic conditions. Cultures were maintained at 25±2°C in continuous light of 2000 lux intensity.

Young leaves of plants were excised into 3-4 parts, their margins were removed and inoculated (after sterilization) on MS (1962) nutrient medium supplemented with 2,4-D (1.0,2.0,3.0,and 4.0 mg/l) and 1.0 mg/l 2,4-D with BAP (1.0,2.0,3.0,and 4.0mg/l). The leaf explant grew and proliferated into callus. The callus raised on 1.0 mg/l 2,4-D with various concentration of BAP (1.0,2.0,3.0and 4.0 mg/l) was more prolific than 2,4-D supplemented medium alone (without BAP). In 25-30 days after inoculation a good amount of callus was formed on supplemented media. Initially the colour of callus on each medium was whitish green and compact in nature. The estimation of selective metabolites was carried out using different protocols. The powdered form of *in vivo* leaf and callus (leaf) were used for analysis of some selective metabolites viz. Protein (Bradford 1976), proline (Bates *et al.* 1973), phenolics (Bray and Thorpe 1954), nitrogen (Snell and Snell 1967) and total sugar (Nelson 1994).

All experiments were repeated thrice and means (± SD) were calculated.

## RESULTS AND DISCUSSION

In the present study, (Fig.1) maximum content of protein was observed in the callus raised on MS +4.0 mg/l 2,4-D (5.82 ± 0.121 mg/gdw) and very much less in *in vivo* leaf (1.86± 0.02 mg/gdw). This indicates that under high concentration of 2,4-D the poorly growing callus is under stress in comparison to the *in vivo* growing leaf and hence the protein might be related to *in vivo* secondary metabolic biosynthetic activity. (Thomson *et al.* 1991) also reported. (The presence of higher protein

level in the plant points towards their possible increase in food value or that protein based bioactive compound could also be isolated in future).

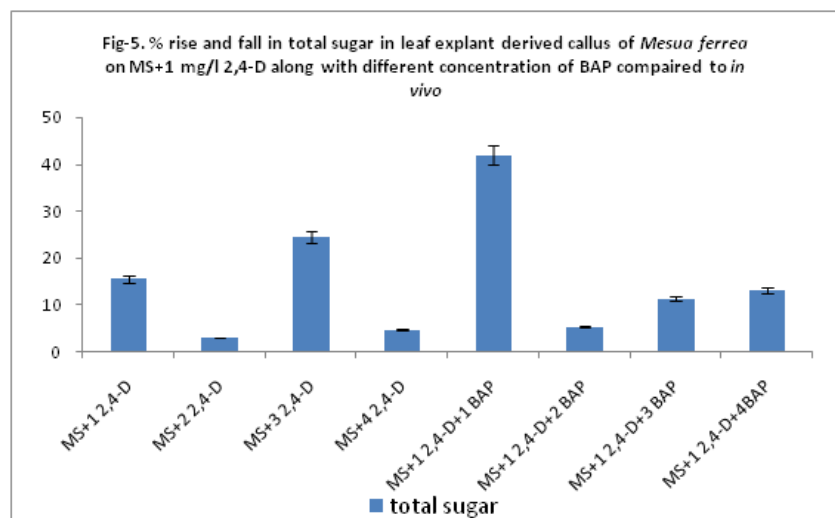
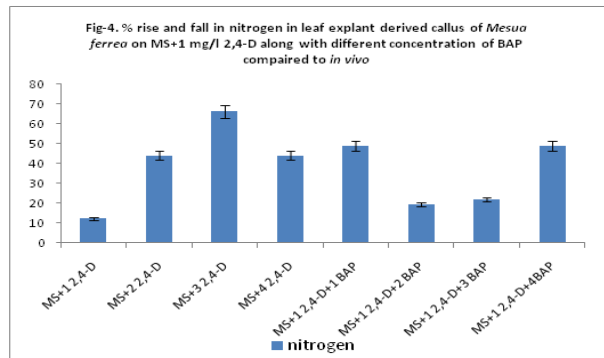
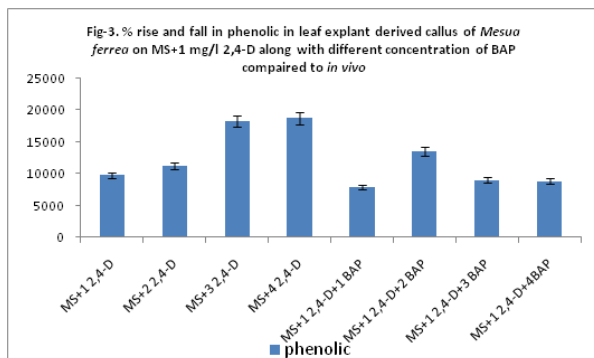
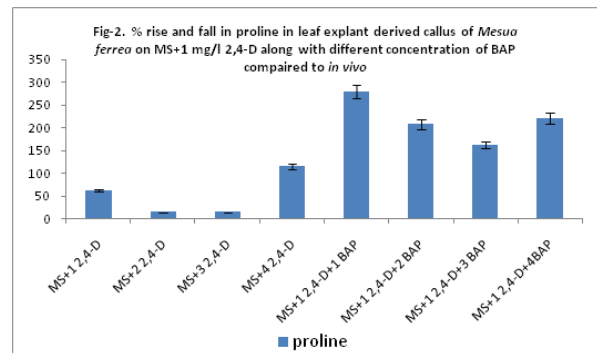
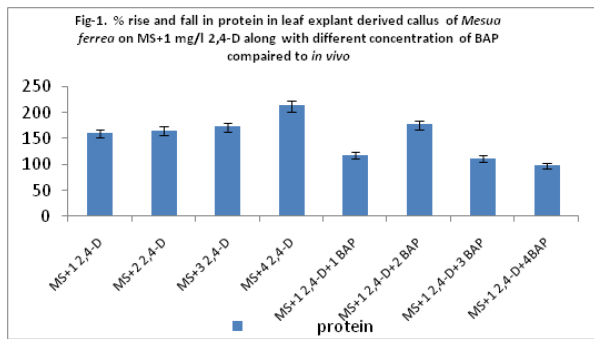
The highest content of proline (Fig.2) was observed in the callus raised on MS +1.0 mg/l 2,4-D,+1.0 mg/l BAP (1.06±0.167 mg/gdw) and minimum in MS +1.0 mg/l 2,4-D (0.108±0.38 mg/gdw). Interestingly, Proline content in callus raised on MS+1.0 mg/l 2,4-D +1.0 mg/l BAP (1.06±0.167 mg/gdw) was much higher than in growing leaf (0.29±0.02 mg/gdw), indicating the calli to be under osmotic stress compatibly adjusted by higher proline biosynthesis (Claussen 2005).

The highest amount of phenolics (*in vitro*) (Fig.3) was observed on MS+4.0 mg/l 2,4-D (101±30.024mg/gdw) and minimum in MS+1.0 mg/l 2,4-D (43.036±10.803mg/gdw) but phenolic content in callus raised in *in vitro* conditions was much higher than *in vivo*, indicating stress combating potential of the callus through antioxidant activity (Abdelrahman *et al.* 2012). Beside high phenolic content serves as medicinal principle too.

The highest amount of nitrogen (Fig.4) was observed in the callus raised on MS+3.0 mg/l 2,4-D (0.675±0.015 mg/gdw) and minimum in MS+1.0 mg/l 2,4-D+4.0 mg/l BAP (0.209±0.005 mg/gdw) but nitrogen content in callus raised on MS+2,4-D (1.0-4.0 mg/l) was higher than *in vivo*. But the callus combats increasing stress with increases in 2,4-D concentration which upon addition of BAP appears to be reduced. Though higher nitrogen content is generally indicative of better growth as part of protein, but in this case higher nitrogen content may also indicative of higher biosynthesis of nitrogenous secondary metabolites such as alkaloids, as the amount of protein declined with increasing PGRs combination concentration in the culture medium. However, the protein still remained higher than *in vivo* control, indicating probably synthesis of proteins for withstanding *in vitro* conditions.

The highest content of total sugar (Fig.5) was observed in the callus raised on MS+1.0 mg/l 2,4-D (2.08±0.087mg/gdw) and minimum in MS+1.0 mg/l 2,4-D+1.0mg/l BAP (0.97±0.800mg/gdw) but sugar content in callus raised on 3.0 mg/l 2,4-D only was higher than *in vivo* condition. Sugars fulfil an extraordinarily wide range of functions in plants. They serve as valuable energy resources that are easy to store and remobilize. They

require for the synthesis of cell walls and carbohydrate polymers. They are also necessary for starch accumulation and serve as precursors for starch accumulation and intermediate. However, in the present experimental set high sugar content with low callus growth is indicative of either osmotic adjustment or stress associated glycoside formation, due to less proliferation and better sustenance noticed (Kishor *et al.* 1990).



## CONCLUSIONS

We report in present investigation that the callus of *Mesua ferrea* plant leaf is a rich source of nitrogenous metabolites and can be used as raw material in pharmaceutical industries, without destroying the plant. So far, this type of tissue cultural work is not reported on this plant.

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