Ascorbic Acid (antioxidant) in *Ailanthus excelsa* and *Balanites aegyptiaca* and Effect of Growth Regulators and Salts on it *in vitro*

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Abstract: *Ailanthus excelsa* and *Balanites aegyptiaca* are two wild growing common plant species of desert, having medicinal importance. Unorganized tissues of these useful plants were established on MS medium supplemented with 1.0mg/L BAP+1.5mg/L 2,4-D and 1.5mg/L BAP +2.0mg/L 2,4-D respectively (standardized MS medium). Parts of established tissues were transferred to standardized (Sd) MS medium fed with various concentrations (1,2,3 mg/L) of growth regulators (IAA,NAA ) and ( 10,20,30mg/L ) salts ( NaCl, KCl ) separately. Tissues at the maximum GI (in all samples) were harvested, dried, powdered and analyzed for estimation of ascorbic acid. Maximum amount of ascorbic acid was calculated in callus fed with 1mg/L IAA, NAA and 20 mg/L NaCl and KCl both in *Ailanthus excelsa* and *Balanites aegyptiaca*.

Keywords: *Ailanthus excelsa*, Balanites aegyptiaca, antioxidants, growth regulators, salts

1. Introduction

**Ardu** (*Ailanthus excelsa*) belonging to family **Simarubaceae** is a large deciduous tree having a lot of medicinal uses. It is a native of India and Sri Lanka. The plant is recognized for its varieties of medicinal uses like contraceptive, post partum treatment, intestine tape worm, dysentery, epilepsy, heart troubles, asthma etc. The plant also having anticerescous, antibacterial, antimicrobial and antifungal activity. Two new tetracyclic triterpenes (ailexcene and ailexcelol) were isolated from the heartwood of *Ailanthus excelsa* which are tested for their antifungal activity (Srinivas et al, 2006).

**Balanites aegyptiaca** “Desert Date” of family **Zygophyllaceae** is a common wild plant found in many kind of habitates, tolerating a wild variety of soil types from sand to heavy clay and climatic moisture levels. It is believed indigenous to all dry lands.

The root, stem bark, fruit pulp and kernel cake of *Balanites aegyptiaca* have medicinal properties. Fruit is used in whooping cough also in leucoderma and other skin diseases. It is used as an oral hypoglycemic and an antidiabetic. An aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in treatment of jaundice. It is also used to treat dysentery and constipation. Fruit is used to treat liver diseases and as a purgative and sucked by school children as a confectionary in some countries.

**Balanites aegyptiaca** contains steroids ( saponins, sapogenins, diosgenins ) used as raw material for industrial production of contraceptive pills, corticoids, anabolisants and other sexual hormones. The saponins occurring in roots,wood chips and fruits facilitate their use for washing clothes.

**Antioxidant** is simply a molecule that prevents another molecule from oxidizing. Since there are many processes of the body which result in oxidation . The intake of antioxidant is essential to counteract some of the negative results of the buildup of too many oxidized molecules in the body.

Primary metabolites are produced as a result of photosynthesis by which green plants utilize solar energy to yield the photosynthetic product-Carbohydrate. Besides this process some other primary synthetic processes also occur in plants which yield certain vital products such as proteins, amino acids, minerals and other nutritive contents, ascorbic acid, lipids, vitamins, nucleotide and energy compounds like alcohols, organic acids etc.

**Ascorbic Acid** or Vitamin C is claimed as a ‘Cure all’ for many human diseases and problems from cancer to common cold. Ascorbic acid is required in synthesis of collagen, neurotransmitters, steroid hormones etc. Vitamin C promotes the healing of wounds, bone fractures, bruises, hemorrhages, bleeding gums and forms the protective barrier between infections or disease and the surrounding healthy tissue. As an antioxidant it has many beneficial functions in combating many diseases and infections and also promotes proper calcium absorption. In plants, ascorbic acid is essential for photosynthetic activity via the detoxification of super oxide and hydrogen peroxide (H₂O₂) in chloroplasts in the absence of catalysts. Thus, it acts as a reducing agent in biological systems. It also assists in healthy cell development as well as normal tissue growth and repair. Vitamin C is a water soluble vitamin.

Free endogenous ascorbic acid production has been reported in tissue culture of *Momordica charantia* and *Emblica officinalis* (Mohan et al.,1974), *Datura metel* and *Datura tatula* (Nag et al., 1974 ), *Trigonella foenum-graceum* (Jain et al., 1975 ); *Ephedra foliata*, *Helianthus annus*, *Agave wightii* and *Tephrosia purpurea* (Khanna et al., 1977 ); *Solanum xanthocarpum* (Manot, 1977), *Atropa belladona* (Sharma, 1977), *Papaver somniferum* (Gaur, 1978, Khanna et al., 1977 ),*Daucus carota* (Sogani, 1978), *Solanum nigrum* (Rathore et al., 1979), *Tribulus alatus* and *Zygophyllum simplex* (Jit et al., 1986), *Lycium bararum*.
A×V ×1000×100W

Ascorbic acid content per 100 gm dry weight was calculated as follows:

Free ascorbic acid = \( \frac{A \times V}{W} \times 1000 \times 100 \)

Where, \( A = Y = \) mg ascorbic acid / ml of original extract
V = total volume of the original extract (in ml)
W = weight of the plant tissue sample (in mg) used for analysis

3. Results and Discussion

Maximum GI was observed at the age of eight weeks in standardized (Sd) MS medium and standaized MS media supplemented with various concentrations (1,2,3 mg/L) of growth hormones (IAA and NAA) and salts (NaCl and KCl at 10,20,30 mg/L) in A. excelsa and B. aegyptiaca . Calli were harvested at maximum GI from all the samples separately in both plants and analyzed for ascorbic acid content.

It was observed that amount of ascorbic acid was increased in callus fed with growth regulators IAA and NAA. Increase was from Sd MS medium to Sd MS medium fed with 1 mg/L but after that amount decreased in Sd MS medium fed with 2 mg/L up to Sd MS medium fed with 3 mg/L IAA and NAA separately in both plant species. The amount calculated in calli fed with 3 mg/L IAA and NAA was even lower than amount of ascorbic acid present in callus grown on Sd MS medium. Maximum amount of ascorbic acid was calculated in callus fed with 1 mg/L IAA and NAA in A. excelsa and B. aegyptiaca ( Table 1.1).

In calli fed with salts, the amount of ascorbic acid was increased from Sd MS medium to calli fed with 10 mg/L KCl, NaCl and from 10 mg/L to 20 mg/L but decreased in 30 mg/L in both plant species. Maximum amount was calculated in calli fed with 20 mg/L NaCl and KCl in A. excelsa and B. aegyptiaca ( Table 1.2).

Growth hormones showed positive response than salts as amount was comparatively higher in calli fed with growth hormones than salts in both plant species. B. aegyptiaca has higher amount of ascorbic acid than A. excelsa in all samples.

References

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Table 1.1: Effect of Growth Regulators on Ascorbic Acid Content (mg/100 g.d.w.) IN A. exselsa AND B. aegyptiaca IN VITRO (AT MAXIMUM GI)

| Name of Plant | Sd MS Medium | Growth Regulators | IAA/L | NAA/L |
|---------------|--------------|-------------------|-------|-------|
|               |              | 1mg               | 2mg   | 3mg   | 1mg  | 2mg  | 3mg  |
| A. exselsa    |              |                   |       |       | 0.40±0.04 | 0.42±0.06 | 0.40±0.04 | 0.36±0.04 | 0.41±0.04 | 0.39±0.04 | 0.35±0.05 |
| B. aegyptiaca |              |                   | 0.46±0.05 | 0.48±0.05 | 0.46±0.06 | 0.42±0.06 | 0.47±0.04 | 0.45±0.07 | 0.40±0.04 |

Values are mean of five replicates ± SD

Table 1.2: Effect of Salts on Ascorbic Acid Content (mg/100 g.d.w.) IN A. exselsa AND B. aegyptiaca IN VITRO (AT MAXIMUM GI)

| Name of Plant | Sd MS Medium | Salts | NaCl/L | 10mg | 20mg | 30mg |
|---------------|--------------|-------|--------|------|------|------|
|               |              | KC/L  |        | 10mg | 20mg | 30mg |
| A. exselsa    | 0.40±0.04    |       | 0.42±0.06 | 0.43±0.06 | 0.36±0.07 | 0.41±0.05 | 0.42±0.04 | 0.35±0.04 |
| B. aegyptiaca | 0.46±0.05    |       | 0.48±0.06 | 0.48±0.06 | 0.43±0.07 | 0.47±0.05 | 0.48±0.05 | 0.42±0.04 |

Values are mean of five replicates ± SD