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# *Comparative study of tissue cultured and wild variety of Safed musli*

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## Abstract

*Bhavprakash* described the *Abhava dravyas* for group of drugs like Jeevaniya gana, *Ashtavarga* etc which were not available that time also, now a day's number of valuable herbs are extincted due to lack of propagation methods. *Safed musali* is one such drug which is used in large quantities by *Ayurvedic*, *Unani* and other traditional practitioners, now it is rare. With the help of biotechnology presently we can propagate the endangered species like Safed musali in some artificial conditions and cultivate them in the natural habitat. Tissue culture technology is one of the effective bio-technological tools to propagate large number of sampling which have exact genetic makeup of the mother plant; this is possible as each and every cell of a plant is *totipotent*. Tissue culture does not much effect or alter the natural characteristics of *Safed musali* but increases the yield.

**Keywords:**

*Safed musali, tissue culture, bio-technology, totipotent.*

## Introduction

*Ayurvedic* system in general consider or cultivated herbs are less potent than which are present in natural habit but the present scenario if one sticks to this principle and go on using such herbs make cause serious problems like extinction of particular species or serious adulteration due to demand and supply therapy, this is not at all desirable and has to be check immediately. The answers for this are

1) Conservation of forest and protecting the biodiversity

2) To encourage suitable cultivation practices with similar climate conditions as the natural herbs have. This will ensure the limiting of indiscriminate collection at the same time ensuring the identity, quality and quantity of required drug. In a long term this methods will also help in conservation of plants

## Role of tissue culture

Tissue culture is an effective biotechnological tool to propagate large number of sampling which have exact genetic makeup of the mother plant this

is possible as each and every cell of a plant is *totipotent* (capable of generating complete plant). Hypothetically we can state that if we select explants with certain Rasa, *Guna*, *veerya*, *vipaka*, qualities. Tissue *culturate* and cultivate it under similar condition at that of the mother plant, it should have similar morphological, biochemical as well as therapeutic quantities as that of it's mother plant and same time maybe better qualities too.

**Aim:**

The aim of the study is to compare morphological, biochemical and *phytochemical* Standards between the wild and tissue cultured variety of *Safed musli*.

**Objective:**

1) To detect the difference between tissue culture and wild variety of Safed musali

2) To increase the production potential of *Safed musali* in terms of quantity and quality .

**Material and method:**

***Micropropagation of Safed musali***.

Tissue culture procedure has been done in Tissue culture department of *Marathwada Krishi Vidyapeeth , Parbhani.*

**A) Tissue culture media**

**Medium -1**

This medium is used for initial bud elongation and shoot multiplication has following composition.

**Macronutrients**

| Components | quantity mg/lit |
|---|---|
| $NH_4NO_3$ | 1650.0 |
| $KNO_3$ | 1900.0 |
| $Cacl_2\ 2H_2O$ | 440.0 |
| $MgSO_4\ 7H_2O$ | 370.0 |
| $KH2PO_4$ | 170.0 |

**1) Micronutrients**

| | |
|---|---|
| KI | 0.83 |
| $H3BO_3$ | 6.20 |
| $MnSO_4\ 4H_2O$ | 22.30 |
| $ZnSO_4\ 7H_2O$ | 8.60 |
| Na2MO4 2H2O | O.25 |
| $CuSO_4\ 5H_2O$ | 0.025 |
| $CoCl_26H_2O$ | 0.025 |
| $NA_2EDTA$ | 37.30 |
| $FeSO_4\ 7H_2O$ | 27.80 |

**2) Vitamins and other supplements**

| Components | quantity mg/lit |
|---|---|
| Inositol | 100.0 |
| Glycine | 2.0 |
| Thiamine HCL | 0.1 |
| Pyridoxine HCL | 0.5 |
| Nicotinic acid | 0.5 |

+ 0.88 µM BAP +0.6 µM Kinetin +10% CW +2% sucrose +0.5 % agar

**Medium -2**

This medium is used for rooting and has following composition.

**Macronutrients**

| Components | quantity mg/lit |
|---|---|
| $KNO_3$ | 80.0 |
| $MgSO_4\ 7H_2O$ | 720.0 |
| $NaH_2PO_4\ H_2O$ | 16.5 |
| $Ca(NO_3)_2\ 4H_2O$ | 300.0 |

**Micronutrients**

| Components | quantity mg/lit |
|---|---|
| KI | 0.75 |
| $H3BO_3$ | 1.50 |
| $MnSO_4\ 4H_2O$ | 7.0 |
| $ZnSO_4\ 7H_2O$ | 2.60 |

**Vitamins and other supplements**

| | |
|---|---|
| Glycine | 3.0 |
| Thiamine HCL | 0.1 |
| Pyridoxine HCL | 0.1 |
| Nicotinic acid | 0.5 |
| Calcium | 1.0 |

| | |
|---|---|
| pentothenate | |
| Cysteine HCL | 1.0 |
| +2% sucrose | 1.0 |

**Explants, it's collection, surface sterilization and inoculation.**

Young buds 10 to 20 mm long with small portions of tubers attached to them are used as explants. sprouting occurs only in a specific season (monsoon) and explants can be collected only in the sprouting season. Mature tubers /roots collected from healthy and disease free plants at harvest time (January t-February )are thoroughly washed, air dried, stocked in glass jars and maintained at room temperature in the laboratory. The tubers/ roots sprouts in May- June and produce young buds. Buds so produced are collected in a 250 ml conical flask containing water( to prevent them from drying) the birds are gently washed off in running tap water for 5 to 10 minutes, treated with an antiseptic detergent (0.5%) *savlon* for 3 to 5 minutes and the antiseptic detergent is washed off thoroughly with many changes of distilled water .

The buds are then taken to the Laminar air -flow cabinet and treated with 0.12% $HgCl_2$ solution for 15 to 20 minutes (with frequent shaking). All traces of $HgCl_2$ are washed off thoroughly in many changes of sterile distilled water. Then water is decanted completely and the buds transferred to a sterile Petri plate (10 to 12 cm). Under aseptic conditions the birds are dissected with a sharp scalped and the outer leafy scales are removed explants are then cut into 2-5 mm size, 5 and incubated on medium.

C**) Incubation**

The cultures are incubated at 28 ± $1^0$ C for 16 hours in light and at14 -15 and ± 1.25 $^0$C for 8 hours in the dark. In about 10 to 15 days the explants sprout and elongate. These shoots can either be transferred to medium-2 for rooting, or aseptically dissected and inoculated again on medium-1. When inoculated on medium-1 they multiply and produce 3-5 shoots in 4 to 6 weeks. The shoots can also be grown in liquid medium (medium-1 without agar).

**D) Rooting**

Shoots (30-50 mm long) produced in medium- 1 are dissected at the time of subculture and placed on filter paper supports kept in medium- 2 with their lower portion touching the medium. Roots develop in 2 to 3 weeks.

**E) Hardening**

Rooted plantlets are removed from culture tubes and washed thoroughly to remove traces of nutrient medium. The plantlets are then planted in a sterile mixture (1:1) soil and vermiculite in small earthen pots. The potted plantlets are maintained at 25 to 28$^0$C and a 12 hours photo period. After hardening the plantlets are transferred to a *polyhouse* followed by transplantation in the field within a month.

**Cultivation of *Safed musali***

For *musali* cultivation purposes red loamy, gravely soil or well drained soil is suitable. Desired PH of soil for cultivation is 6-7. Rain fall in the range of 50-75 cm is favorable for cultivation. Cultivation done by following methods.
Plantlets and roots are placed at a distance of 20×20 cm.

**Collection**

In month of February tuberous roots in the form of bunches are uprooted by digging method and no mechanical

method is suggested as it may result in loss of roots. Roots are collected and spread in shade for 4-8 days. Then fleshy roots are separated from bunches. These are then dried under shade. The outer yellowish layer is easily separated from the in inner white part. So the roots get decorticated. Impression

1) Irrigated and *manured* plot gives highest yield.
2) Tissue culture plants give yield 30 percent more than wild plants. Preservation and storage the dried root should be stored in a dry place, preferably in air tight contain.
3) Comparative study of tissue cultured and wild *Safed musali*

| Sr no. | Name of plant | leaf | | | Root | | |
|---|---|---|---|---|---|---|---|
| | | number | Length | color | Number | Length | color |
| 1 | Tissue culture | 12-22 | 10-18 inches | green | 10-16 | 9-12cm | brown |
| 2 | wild | 12-16 | 10-16 | green | 6-10 | 3-9cm | brown |

**Moisture content of Safed musali r**oot

| Sr. no. | Name of sample | Initial weight | Wt afterwards | loss | percentage |
|---|---|---|---|---|---|
| 1 | Tissue culture | 10.0012 | 9.121 | 0.8802 | 8.802 |
| 2 | wild | 10.0002 | 9.201 | 0.7992 | 7.992 |

**Total ash of *musali* root powder**

| Sr. no. | Name of sample | Sample no. | Wt of the crucible (before) $W_1$ | Wt of the crucible (after) $W_2$ | Wt of the ash $W=W_2-W_1$ | Percentage of ash in % |
|---|---|---|---|---|---|---|
| 1 | Tissue culture | I | 10.2964 | 10.3211 | 0.0247 | 2.4 |
| | | II | 10.2964 | 10.3218 | 0.0254 | 2.5 |
| | | III | 10.2965 | 10.3224 | 0.0260 | 2.6 |
| | | Average | | | | 2.5 |
| 2 | Wild | I | 11.4708 | 11.4996 | 0.0288 | 2.8 |
| | | II | 11.4710 | 11.4988 | 0.0278 | 2.7 |
| | | III | 11.4708 | 11.4982 | 0.274 | 2.7 |
| | | average | | | | 2.7 |

**Thin layer chromatography of *musali* root powder:**

Absorption layer: silica gel

Samples: alcohol extracts of the samples

**Thin layer chromatography of Tissue cultured *Safed musali:***

| Extract | Solvent system | Spray/treatment | No. of spots | Rf values |
|---|---|---|---|---|
| alcohol | Toluene: diclorometh ane: methanol (7:3:1) | Exposed to iodine vapours | 3 | 0.1,0.46,0.54 |
| alcohol | Chloroform: methanol(95:5) | Exposed to iodine vapours | 4 | 0.2,0.28,0.84,0.87 |

**Thin layer chromatography of wild Safed musali:**

| Extract | Solvent system | Spray/treatment | No. of spots | Rf values |
|---|---|---|---|---|
| alcohol | Toluene: diclorometh ane: methanol (7:3:1) | Exposed to iodine vapours | 3 | 0.1,0.46,0.54 |
| alcohol | Chloroform: methanol(95:5) | Exposed to iodine vapours | 4 | 0.2,0.28,0.84,0.87 |

**Summary:**

Qualitative tests has been performed in Drug Testing Laboratory at Government *Ayurved* College, Nanded, and chrome laboratory, *Mulund*, Mumbai, Maharashtra.

1. there is a difference in between tissue cultured *musali* and wild *musali* i.e. the number of roots of T.C. *musali* is 10-16 & length is 9-12cm while in wild *musali* number of roots areis 6-10 and length of root is 3-9 cm.
2. Moisture content in T.C. *musli* is 8.802%while in wild musali is 7.992%.
3. Total ash value of T.C. *musali* is 2.5% & of wild *musali* is 2.7%.
4. Water soluble ash value of T.C. *musali* & wild *musali* is 1.0%. and 0.9% respectively.
5. Acid insoluble ash value of T.C. musali & wild musali is the same i.e.1.0%.
6. There is some difference in water soluble extraction of T.C. *musali* and wild musali is 2.0% which is same in both varieties.
7. Petroleum ether soluble extractive is near about same. i.e. 0.79% in T.C. *musali* & 0.76% in wild *musali*.
8. As per T.L.C. studies the number of spots and Rf. Values of T.C. *musali* and wild musali are same, example in toluene: dichloromethane: methanol (7:3:1) mobile phase both varieties shows 3 spots of exactly same Rf values i.e.0.1,0.46, 0.56.
9. On changing the mobile phase of T.L.C. i.e. chloroform :methanol (95:5) both varieties shows 4 spots of same *Rf* values as 0.2,0.28, 0.84,0.87.
10. UV-VIS *spectro* photometric reports shows that absorption readings are similar on large extent but different in small scale.
11. In HPTLC tests with mobile phase toluene: *ethylacetate*: *formicacid* (5:4:0.5) T.C. *musali* shows 6 spots while wild *musali* shows 7 spots. Here the maximum spots shows similar *Rf* values with similar height. The absorption graphs also shows similar pattern.

## Conclusion:

***Need is the mother of invention.***

Adopting new technology for long term benefits is always desirable much so far science like *Ayurveda* considering the importance of supply of quality herbs and to prevent threat of, deforestation mass propagating techniques like tissue culture are essential. *Safed musli* is one such drug which is used in large quantities by *Ayurvedic*, *Unani* and other traditional practitioners. There are many types of drugs which are available in the name of *Safed musli* and the study clearly reveals that *chlorophytum borivilianum* is most widely marketed variety. tissue culture, cultivation has made this drug more available with uniform quality throughout the year making it a popular choice of cultivated medicinal plants among many farmers considering this impact it was decided to take up a comparative study of various aspects like morphology biochemical and *phytochemical* study. The main advantages of *micro propagation* are that cultures can be initiated from very small segment from the mother plant, space required is comparatively less, propagation is done under aseptic conditions, and not much care is required in between sub-cultures and in most of the species, *propogationbis* possible throughout the year.

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