

**EFFECT OF CULTURE MEDIA AND
IRRADIATION ON MICROPROPAGATION
AND ANATOMICAL STRUCTURE OF
Brunfelsia pauciflora PLANT.**

By

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APPROVAL SHEET

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ABSTRACT

The aim of this study was to investigate the most suitable protocol for micropropagation of *Brunfelsia pauciflora* plant, as affected with type and strength of culture media supplemented with different growth regulators and study the effect of gamma irradiation on shooting behavior, rooting, acclimatization, chemical analysis, Anatomical studies and genetic fidelity of *Brunfelsia pauciflora* shoot.

Micropropagation of *Brunfelsia pauciflora*: application of a sodium hypochlorite NaOCl (0.75%) for 20 min then 3 g/l mercuric chloride (HgCl₂) for 1min. gave the best disinfection and the highest survival percentage (86.67%) of during surface sterilization. The sampling data in July resulted in the highest value for the number of shoots and tallest shoots on establishment stage. Using full MS medium supplemented with 3.0 g/l AC resulted in the highest shoot length, number of shoots performed and the highest value of leaves number. On multiplication stage using MS medium supplemented with 2mg/l of BA produced the maximum number of proliferated shoots while MS medium supplemented with 4mg/l BA gave the highest callus formation. The highest mean value of shoot length and number of leaves was recorded from using MS medium supplemented with 0.5 mg/l TDZ and 2.0 mg/l 2iP. For shoot regeneration from leaf explants, MS medium supplemented with 0.5 mg/l TDZ and 2.0 mg/l 2iP resulted the highest mean value of callus formation, shoots regeneration, shoot length and number of leaves. The highest values for number of roots and root length were recorded with 2 mg/l IBA. The highest value of flowers for shoots obtained under temperature degree at 22 °C and light intensity at 1500 lux after 12 weeks on MS medium supplemented with 2 mg/l IBA plus 3.0 g/l AC. The maximum percentage of survival of acclimatized plantlets (96.67%) was recorded from using a mixture of peatmoss plus perlite at 2:1 (v/v) produced the highest survival percentage, plantlet length and number of leaves.

Irradiation on *Brunfelsia pauciflora* plant with gamma rays resulted as a change in chemical and anatomical structure of shoot tips. Shoot tips were exposed to gamma irradiation at 0, 5 and 10 Gy as gave 100% of survival percentage during *In vitro* multiplication. The highest values for number of shoots, number of leaves and the longest shoot were recorded after multiplication stage with exposure to 10Gy. The shoots exposed to gamma rays at the dose of 10 Gy gave the maximum values for number of roots, root length, number of leaves and the longest shoot at *In vitro* rooting. Application of gamma rays at 10 Gy gave vigorous growth with healthy appearance at acclimatization stage. Moreover it resulted in the greatest of leaf area (cm²), stem diameter (mm), fresh and dry weights of shootlets, pigments content of chlorophyll (a and b) and the lowest content of carotenoids, produced the highest chemical contents on N. P. K. elements, total carbohydrate, soluble sugars, total free amino acids, flavonoid, indolic but the lowest content of phenolic and Total Alkaloid compounds was recorded with gamma rays at 10 Gy. Stem thickness exhibited an increase due to both doses of 5 and 10 Gy and reached maximum values over the control. However, the thicknesses of both upper and lower epidermis of leaves were only increased at the rate of 5 Gy over control. The true to tip between mother plant and the micropropagated plants only (sample number 1: control) had been confirmed. Between mother plant and irradiated plants by gamma rays at the doses of (5, 10 and 20 Gy) resulted in the DNA polymorphism in the collection of genotypes analyzed, and generated many polymorphic markers ensuring a good coverage of the genome. The ISSR profiles of plantlet irradiated with gamma rays of treated plants with radiation at the rate of 10 Gy produced new fragments, however subjecting the plants to gamma rays at the rate of 20 and 30Gy caused full damage for DNA and death of plants.

Key words: Micropropagation, growth regulators, Irradiation, Gamma rays, *Brunfelsia pauciflora*, ISSR.

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LIST OF ABBREVIATIONS

AC	Activated charcoal
ABA	Abscissic acid
ADS (AdSO ₄)	adenine sulfate
B5	Gamborg medium
BA (BAP)	Benzyladenine or 6-benzylamino purine
CM	coconut milk
Colorx	5.25% sodium hypochlorite (commercial bleach)
cv (s)	cultivar (s)
DNA	Deoxyribonucleic acid
GA ₃ (GA ₇)	Gibberellic acid
HgCl ₂	mercuric chlorid
IAA	Indole acetic acid
IBA	Indole butyric acid
2,4-D	2,4-dichlorophenoxy acetic acid
2iP	2-isopentenylamino purine
Kin	Kinetin (6-furfurylaminopurine)
LS	Linsmaier & Skoog medium
M	Molar = Molecular weight g/l
μM	Micro mol
mM	Milli mol
MS	Murashige & Skoog medium
NAA	Naphthalene acetic acid
NaOCl	sodium hypochlorite
ppm	Part per million
PVP	Polyvinyl pyrrolidone
Tween 20	Polyoxyethylene sorbitan monolaurate
TDZ	thidiazuron
v/v	volume by volume
WPM	Woody Plant medium
Zeatin	[6-(4-hydroxy-3-methylbut-2-enylamino)-purine]
°C	Degree Celsius
μ	micro(10 ⁻⁵ m)
μm	micrometer (10 ⁻⁶ m)
bp	Base pair
cm	Centimeter
D.W.	Dry weight
DNA	Deoxyribo nucleic acid
F.W.	Fresh weight
Fig.	figure
Gy	Gray
Kr	kilo rad
LSD	Least significance difference
mg	Milligram (s)
mg/l	Milligrams per liter
Min.	minute
Mw	Molecular weight
PCR	Polymerasa chain reaction
ISSR	Inter Simple Sequence Repeats
γ	Gamma ray
LD ₅₀	a 50% of irradiated explants died
Co ⁶⁰	Cobalt-60 source

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