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Efficient Plantlet Regeneration from Leaf Base Explants of Aloe Vera (L.) Burm.F. Var. Cim-Sheetal an Important Medicinal Plant

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Abstract: *The Present Study Was Undertaken To Investigate The Possibility For Developing A One-Step Regeneration Protocol In Aloe Vera (L.) Var. Cim-Sheetal. For This, The Effect Of Leaf Base Explants Was Cultured On Ms Medium Containing Various Concentrations Of Cytokines Bap (0.5-3.0 Mg/L) And Naa (0.5-3.0 Mg/L) Combinations With Bap (1.5 Mg/L). Maximum Number Of Shoot Bud Proliferation Was Observed At (1.5mg/L). Bap, Compared To All Other Concentrations Of Naa (0.5-3.0mg/L) Combinations With Bap (1.5 Mg/L). As The Concentration Was Increased Above (2.0mg/L) Bap, Naa (2.0 Mg/L) Combinations With Bap (1.5 Mg/L) The Shoot Bud Induction Was Reduced Gradually In Bap Individually And Combinations With Naa (2.0mg/L). High Frequency Of Shoots Was Induced At (1.5 Mg/L) Bap. The In Vitro Regenerated Shoots Produced More Number Of Roots On Ms Medium Containing (1.0 Mg/L) Iba. Thus The Plant Developed In Vitro Using Leaf Base Explants Cultures Were Established In Pots Containing Garden Soil Outside Under Shade In Wound Temperature And Light Conditions. These Plants Flowered After 8 Weeks Following Transfer To Pots. The Protocol Established Can Be Used For Rapid Multiplication Of The Specific Producing True To Type Plants. Abbreviations: Bap- 6-Benzylaminopurin, Naa- Napthalen Acetic Acid And Iba- Indole-3 Butyric Acid.*

Keywords: *Aloe Vera (L.) Var. Cim-Sheetal, Multiplication, Leaf Base Explants, Plantlet Regeneration Bap, Naa And Iba.*

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I. Introduction:-

Aloe Vera (Liliaceae), Is A Succulent Plant Indigenous To Northern Africa And Mediterranean Countries And Has Become Naturalized Almost In All Parts Of India (Klein Et Al., 1988). The Plant Has Stiff Gray-Green Lance-Shaped Leaves Containing Clear Gel In A Central Mucilaginous Pulp. A. Vera Has Been Used For Several Thousands Of Years In Folk Medicine In Many Cultures From Ancient Egypt, Greece, And Rome To China And India (Kemp Et Al., 1999). Some Of The Most Important Pharmacological Activities Of A. Vera Are Antiseptic (Capasso Et Al., 1998), Anti Tumor (Winter Et Al., 1981), Anti-Inflammatory (Yagi Et Al., 1998), Wound And Burn Healing Effect (Heggers Et Al., 1993), Anti Diabetic (Rajasekaran Et Al., 2006) And As An Adjunct To Cur-Rent Aids Therapy (Mc Daniel Et Al., 1990).

A. Vera Propagates Vegetative In Its Natural State. However, Propagation Rate Is Very Slow Because A Single Plant Can Produce Only Three To Four Lateral Shoots In A Year. Moreover, The Production Of Aloe Leaves Is Insufficient To Meet The Industry Demand In India (Aggarwal And Barna 2004) And The Production Of Cosmetics, Foods And Pharmaceuticals Containing A. Vera Has Experienced A Slow Increase Due To Limited Availability Of Raw Material With High Quality (Campestrin Et Al., 2006). Therefore, There Is A Need To Develop Suitable, An Alternative Method For Traditional Propagation Of A. Vera.

In Vitro Techniques Using Micropropagation And Tissue Culture Offer A Great Possibility To Overcome This Problem. Micropropagation Using Stem And Lateral Shoot Pieces Of A. Vera Has Already Been Proved Successful (Natali Et Al., 1990; Roy And Sarkar 1991; Meyer And Staden 1991; Aggarwal And Barna 2004). However, Source Of Explants, Their Sterilization Procedure, Media Composition, Culture Conditions, Phenolic Browning Of Explants And Media Discoloration Greatly Affect Shoot Regeneration From Different Genotypes Of The Same Species. A. Vera Exudes Lot Of Phenolic Substances Into The Culture Media Which Could Decrease The Survival Of Explants (Roy And Sarkar 1991). Concentration Of Phenolic

Compounds May Vary In Different Genotypes Of The Same Species (Glynn Et Al., 2004), And Also Those Were Grown Under Different Climatic Conditions (Kjaer Et Al., 2001). Hence Culture Conditions Are Needed To Be Modified Accordingly To Achieve The Desirable Targets. Thus, Present Study Aimed To Develop A Rapid And High Frequency Shoot Regeneration Protocol For Elite Plants Of A. Vera Suitable For Mass Propagation By Improving Culture Media While Controlling Phenolic Browning Of Ex-Plants. Though Direct Efficient Plantlet Regeneration Studies Have Been Conducted So Far, This Paper Deals With The Direct Plant Regeneration System With Large Number Of Shoots Within A Short Period From Leaf Base Explants Of A.Vera Induced By Bap And Naa In Combination With Bap

II. Methodology

Plant Materials Of *Aloe Vera* (L.) Var. Cim-Sheetal Were Collected From One Single Plant Grown In The Department Of Botany Hyderabad. The Leaf Base Were Washed In Running Tap Water For 5 Min, Treated With 2-3 Drops Of Tween- 40 For 10 Min And Finally The Cutted Suitable Leaf Base Parts Were Washed Thrice With Sterile Distilled Water Followed To Subsequently Surface Sterilized With 0.1% $Hgcl_2$ For 5 Min And Washed Thrice With Sterile Distilled Water. The Surface Sterilized Suitable Leaf Base Were Cultured On Murashige And Skoog (1962) Medium Supplemented With 3% Sucrose And Solidified With 0.8% Agar (Himedia) (Ashok *Et.Al.,* 2002) Ph Of The Medium Was Maintained At 5.8 Samples Were Grown At A Photoperiod Of Photo Period Under White Fluorescent Light Of 40-60 Mmol M^{-2} S^{-1} Intensity. For Efficient Plantlet Regeneration The Leaf Base Explants (0.8–1.0 Cm^2) From 8-Week Old In Vivo Seedlings Were Excised, These Explants Were Inoculated To Ms Medium Supplemented With Various Concentrations Of Cytokinins And Cytokines In Combination With Auxins (0.5- 3.0 Mg/L) Of Bap And (1.5 Mg/L Bap) + (0.5-3.0mg/L) Naa (Table-1-2) All The Leaf Base Explants Growth Regulators Were Used As Cytokines Alone In Culture Media. All Media Were Adjusted To Ph 5.8 Before Addition Of 0.8% Agar Agar And Autoclaved At 121^oc And 103 K Pa For 20 Minutes Cultures In 25 X 150 Mm Cultures Tubes.

III. Culture Media And Culture Conditions:

The Defoliated Shoot Explants Of *Aloe Vera* (L.) Var. Cim-Sheetal Measuring 1 To 1.8 Inches Were Inoculated On Ms- Medium Supplemented With Bap, Individually And Their Combinations Like Bap+Naa (Plate 1, Fig1-8). The Ph Of The Media Was Adjusted To 5.8 Either With 0.1 N Hcl Or 0.1n Na Oh, Solidified With 0.8% Difco-Bacto Agar And Autoclaved At 121^oc Under 15 Psi For 15-20 Minutes. All The Cultures Were Incubated At 25^oc With 16h Photo Period Under White Fluorescent Light Of 40-60 Mmol M^{-2} S^{-1} Intensity. The Shoots Proliferated From Leaf Base Explants They Were Excised And Cultured On A Rooting Medium Consisting Of Ms Medium Supplemented With Different (0.5-3.0 Mg/L) Iba. The Rooted Plantlets Were Gently Removed From The Flasks And The Roots Were Washed In Tap Water To Remove Traces Of Agar. The Fresh Nature Of The Explants, The Initiation Of White Compact Callus And Development Of Light Green Or Dark Green Shoot Buds Was Taken As Indication Of The Positive Response (Plate- 1). Ms- Basal Medium Without Any Hormones Served As The Control. The Shoot Buds Developed Into Leafy Shoots Which Grew Further Into Plantlets.

IV. Acclimatization

Plants With Roots Were Transferred During Two Weeks, After Washing Of The Agar With Distilled Water And To Pots With A Mixture Of Soil Rite (1:1). Potted Plantlets Were Covered With Transparent Polythene Membrane To Ensure High Humidity And Watered Every Three Days With Half Strength Ms Salts Solution For Two Weeks In Order To Acclimatize Plants To Field Conditions. After Two Weeks The Acclimatized Plants Were Transferred To Pots Containing Normal Garden Soil And Maintained In Greenhouse Under Natural Day Length Conditions.

V. Results

The Results Of The Leaf Base Explants Cultures On The Development Of Multiple Shoots And Roots Are Shown In (Table-1&2). The Leaf Base Explants Of *A. Vera* Cultured On Different Hormonal Combinations Showed Varied Results.

The Leaf Base Explants Became Active With In Weak After Inoculation And New Shoots Became Distinct By The Seconds And Third Weak With Leaves And Internodes. The Explants Survival From Leaf Base Explants Of Nature Plant Of *A. Vera* Varied With Season. According To The Present Observations, The Explants Were Collected From Field Grown Plants Thought Out The Year To Determine The Ideal Season For Culture Established.

Table-1: Effect Of Bap On Induction Of Multiple Shoots From Leaf Base Explants Of *A. Vera*

Ms + Bap (Mg/L)	% Of Shoot Number	No. Of Shoots Produced	Shoot Length (Cm)
0.0	55	10	2.18
0.5	65	20	5.14
1.0	70	22	7.092
1.5	85	31	7.124
2.0	60	18	6.27
2.5	40	6	4.53
3.0	40	6	5.140

Fig-I Percentage of shoot number on BAP

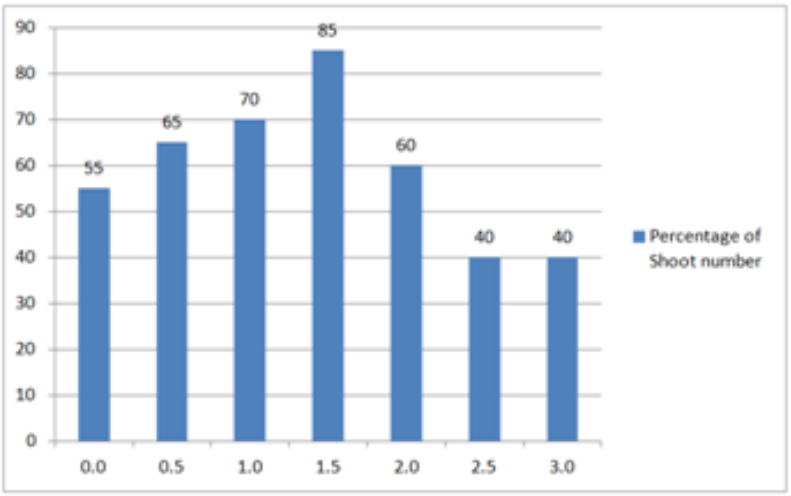


Fig-II Number of shoot produced on BAP

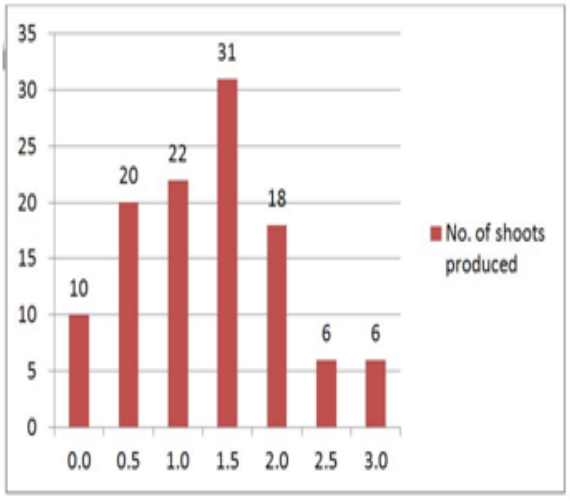
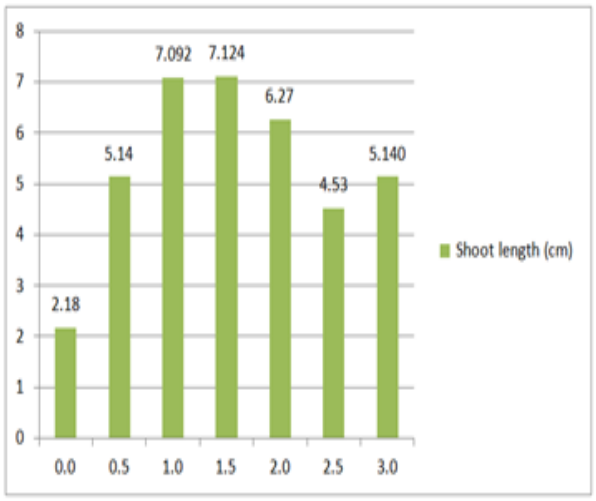


Fig-III shoot length in centimeters on BAP



Effect Of Bap On Multiple Shoot Induction

The Leaf Base Cultured On Ms Modified Medium Supplemented With (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 And 3.0 Mg/L) Bap Showed Maximum Percentage (85%) Of Responding Cultures At (1.5 Mg/L) Bap. At Higher Concentration Of Bap (3.0 Mg/L) The Percentage Of Response Was Reduced Gradually Up To (40%) Respectively. Likewise Maximum Number Of Multiple Shoots Was Found At (1.5 Mg/L) Bap When It Was Added Alone To The Medium, But Highest Percentage Of Responding Cultures And More Number Of Shoots 31 Were Recorded With (7.14 Cm). As The Concentration Of Cytokinin Increased Up To (1.5 Mg/L) Bap The Frequency Of Number Of Shoots Induction Was Found To Be Decreased (18, 6 And 6 At 2.0,2.5 And 3.0 Mg/L) Bap. (Fig 1-4). (Plate-I).

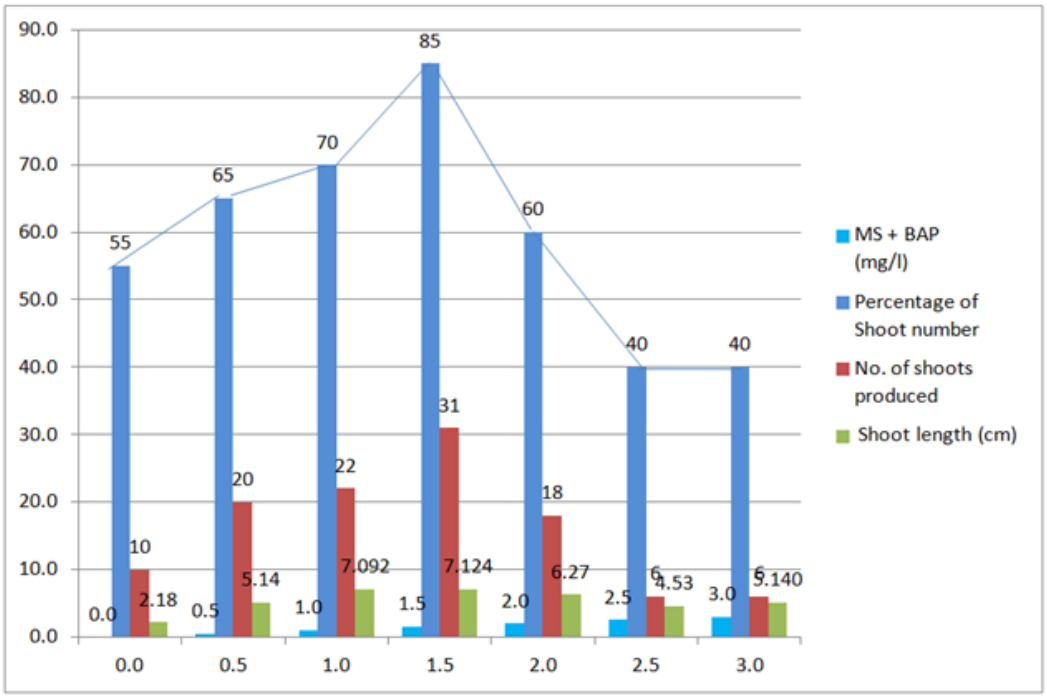


Fig-Iv Percentages Of Shoots Induction, Number Of Shoots And Shoot Length On Bap

Effect Of Naa And Bap On Multiple Shoots Induction

Direct Shoots Proliferation Was Also Found In All The Concentrations Of Bap (1.5 Mg/L) In Combination With (0.5-3.0 Mg/L) Naa Added To The Ms Modified Medium. More Number Of Adventitious Shoots Per Explant Was Recorded At (1.5 Mg/L) Naa + (1.5 Mg/L) Bap Compared To All Other Concentrations Of Naa Used. Low Concentrations Of Naa (0.5-1.0 Mg/L)+ Bap (1.5 Mg/L) Induced Less Number Of Shoots/Explant (12 And 18) But Gradually The Shoots Induction Was Found To Be Decreased Up To Naa (2.0-3.0 Mg/L)+ (1.5 Mg/L) Bap (16,14 And 12) With (75,70 And 65%) Of Responding Cultures And Maximum Number Of Shoots (20) Were Developed On Ms Modified Medium Supplemented With (1.5 Mg/L) Naa + 1.5 Mg/L Bap. (Plate-I) (Fig-3).

Table-2: Effect Of Bap (1.5 Mg/L) +Naa (0.5-3.0 Mg/L) On Induction Of Multiple Shoots From Leaf Base Explants Of *A. Vera*

MS + BAP+NAA (mg/l)	Percentage of Shoot number	No. of shoots produced	Shoot length (cm)
0.0	60	10	2.183
1.5+0.5	65	12	4.043
1.5+1.0	80	18	5.827
1.5+1.5	85	20	6.682
1.5+2.0	75	16	5.702
1.5+2.5	70	14	4.785
1.5+3.0	65	12	4.044

Fig-V Percentage of shoot number on BAP+NAA

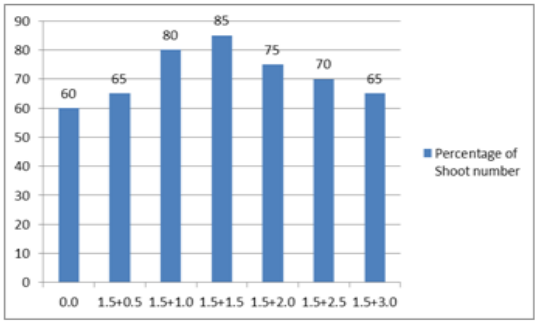


Fig-VI Number of shoot produced on BAP +NAA

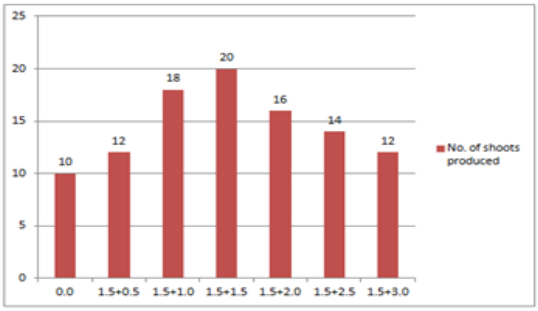


Fig-VII Shoots length in centimeters on BAP+NAA

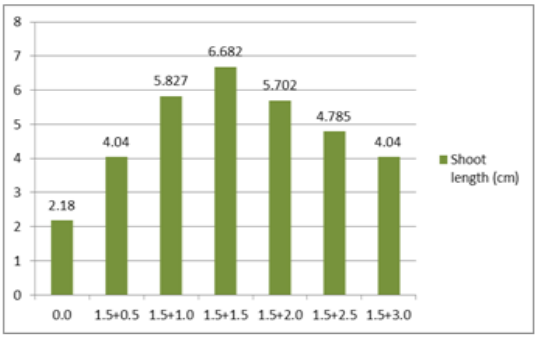


Fig-VIII Percentages of shoots induction, number of shoots and shoots length on BAP +NAA

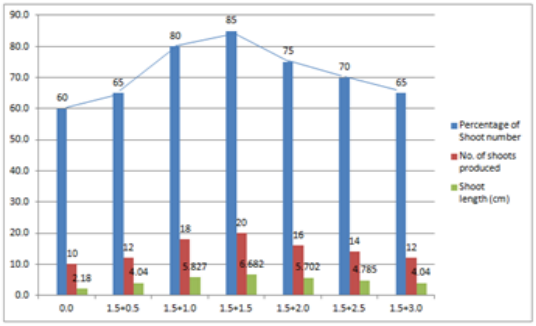


Figure 1 (A-F): Efficient Plantlet Regeneration From Leaf Base Explants Of *Aloe Vera* (L.) **A)**: Defoliated Shoot Base Explant On Ms + Bap 1.5 Mg/L; **B)**: Ms +Bap (1.5 Mg/L) Clusters Of Green Multiple Shoot Buds Formed And A Maximum Of 31 Shoots Developed; **C)**: On Ms + Bap (1.5 Mg/L) Shoot Proliferation From The Base Of Explants, After Third Sub Culture **D):** Up To Nine Roots Formed On Ms + Naa (0.5 Mg/L **E)**: Prior To Final Transfer To Soil Shifted To Big Pots Along With These Net Pots And Polybags; And **(F)**: After Transplanted In Soil.

VI. Discussion

A Number Of Factors Such As Genotype, Culture Medium (Including Growth Regulators And Their Combinations), Physical Environment, Explant Develop-Mental Stage, Etc., Affect Adventitious Shoot Regeneration From Tissue Cultured Explants (Qu Et Al., 2000). Therefore, Present Study Attempted To Optimize The Growth Regulator And Their Concentration For Efficient Direct Shoot Regeneration From Leaf Base Explants Of A. Vera While Controlling The Phenolic Browning. Moreover, The In Vitro Regeneration Of Direct Adventitious Shoots Is An Essential Component To Produce Plants From Elite Materials As To Avoid Formation Of Somaclones

We Were Successful In Direct Regenerating Plants From Leaf Explants Of A.Vera Cultures On Ms Medium Fortified With Different Concentrations Of Cytokinins I.E. Bap (0.5-3.0 Mg/L). Maximum Number Of Shoot Buds Was Induced At (1.5 Mg/L) Bap In Comparison To Bap (1.5mg/L) And Combination With (0.5-3.0 Mg/L) Naa As Role Growth Regulators. It Was Interesting Find Out That The Shoots Induction Was Enhanced In All The Concentrations Of Cytokinins. However The Shoot Bud Proliferation Was Found To More On (1.5 Mg/L) With Bap Compared To (1.5 Mg/L) Bap +(1.5 Mg/L) Naa But The Increasing Concentration Of Bap Up To (1.5 Mg/L) Induced Decreasing Number Of Plantlet Regeneration Among All Hormonal Concentrations Were Used In A.Vera.

Bap And Bap In Combination With Naa Were Selected For Shoot Regeneration/Multiplication In The Present Study As They Are Among The Growth Regulators Used Most Often For The Shoot Organogenesis (Datta Et Al., 2006). Al-Though Shoot Amplification Occurred In Almost All The Hormone Combinations, Bap Alone Was Less Favorable For Shoot Induction. Nevertheless, It Had Been Favorable In Previous Studies (Aggarwal And Barna, 2004) With Shoot Tip Explants. Nevertheless, Bap Was Not Detrimental To Expalnts As Reported By (Meyer And Staden 1991) And (Natali Et Al.,1990) With Decapitated Shoot Explants Since About 85% Of Shoot Frequency And 31 Number Of Shoots Per Ex-Plants Were Observed In The Present Study With Bap (1.5 Mg/L). However, A Combination Of Bap And Naa Was Crucial For Direct Shoot Regeneration.

According To Chaudhuri And Mukundan (2001), The Best Medium For Shoot Induction From Shoot Tips Of Aloe Vera Was Ms Supplemented With (10 Mg/L) Bap + (160 Mg/L) Adenine Sulphate And (0.1 Mg/L) Iba However, This Is In Contrast To The Results Obtained In The Present Investigation As When Bap At A Higher Concentration (3.0 Mg/L) Either Alone Or In Combination With Naa Was Added, It Reduced The Shoot Production And Increased The Number Of Abnormal Shoots. When There Is High Cytokinin Level Present In The Medium, It Causes Cytogenetic Instability (Qu Et Al., 2000) Thus Unsuitable For Clonal Propagation. In Order To Obtain Desirable Clonal Fidelity, Cytokinins Must Be Used At Levels That Stimulate Adventitious Shoots Thereby Avoiding Potential (Aggarwal And Barna 2004) Proved That Aloe Vera Could Be Cultured In Vitro Using Axillary Buds And Ms Medium Containing (1.0 Mg/L) Bap And (0.2 Mg/L) Iba. Citric Acid At (10 Mg/L) Improved The Shoot Multiplication. However, They Could Obtain Maximum Of 5 Shoots Per Explant After 4 Weeks Of Culture. In The Present Study, A Medium Containing (1.5mg/L) Bap Increased The Shoot Number Up To 31per Explants And When Shifted To Sub Culture On Ms +(1.5mg/L) Bap Number Of Shoots Per Explant Was Increased To 35 Per Explants And Their Elongation Was Also Better. This Is The Highest Number Of Shoots Per Explants Reported From A. Vera Compared To Previous Studies With Different Explant Sources And Media Compositions.

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