V- SUMMARY

This investigation was carried out at the Tissue Culture Unit, Horticulture Department, Faculty of Agriculture Moshtohor, Benha University during the period 2004 to 2007.

Explants (shoot tip and one node cuttings) for Marjoram while leaf discs, apical bud segments and corm pieces for Meadow saffron were subjected to running water for 10 minutes then sterilized by using 10% Clorox (Commercial bleach) with two drops of Tween-20 for 10 minutes and washed 3 times with sterilized distilled water for 5 minutes. Different explants were cultured on different medium types, different antioxidant treatments and preculture cold treatments as well as different additives for both Majorana and Meadow saffron were evaluated during establishment stage. However, BAP concentration, 2,4-D concentration and different hormonal balance were tested during indirect regeneration of Meadow saffron. Cytokinin type and BAP concentration were studied on marjoram only during proliferation stage. Moreover, medium strength, GA_3 concentration, auxin type and different concentrations of IBA were evaluated on marjoram only during rooting stage.

The obtained results can be summarized as follow:

5.1. Marjoram (direct regeneration):

5.1.1. Establishment stage:

1. Murashige and Skoog showed the most superior medium as it reduced necrosis and browning while

increased explant development, and greening parameters.

2. Shoot tip surpassed one-node cutting in improving all parameters under study (necrosis, browning and explant development).

3. Combined treatment (anti-oxidant solution and addition of PVP to the culture medium) was valuable in reducing accumulation of phenolic compounds to the lowest level and in turn improved all parameters under study.

4. Exposing of the explant to preculture cold treatment for 3 days (keeping in refrigerator at 5°C) encouraged the best explant development parameters (reducing necrosis and browning while improved both explant development and greening parameters).

5. Supplementation of the culture medium with adenine sulphate was effective in improving of the explant development, greening growth while reduced necrosis and browning.

5.1.2. Proliferation stage:

1. BAP was superior in enhancing proliferation while kinetin was effective in improving growth and greening parameters.

2. Adding 2.0 mg/L BAP to the culture medium was effective in maximizing proliferation while the low BAP concentration induced low necrosis and growth parameters.

5.1.3. Rooting stage:

1- Using of half Murashige and Skoog medium strength enhanced the highest shoot elongation, greening and proliferation parameters.

2- Addition of higher concentration (4.0 mg/L) from GA_3 to the culture medium of the cultured proliferated shoots improved shoot elongation, greening and rooting parameters.

3- Supplementation of the culture medium with indole-3-butyric acid (IBA) enhanced rooting while both IAA and NAA increased growth and greening parameters.

4- Using higher concentration of IBA (2.0 mg/L) in the culture medium maximized rooting while lower concentration (1.0 mg/L) enhanced growth parameter.

5.2. Indirect regeneration (Meadow saffron):

5.2.1. Callus production:

1. Murashige and Skoog was more preferable than both Nitsch & Nitsch and Woody plant medium in increasing explant development and callus production.

2. Apical bud segments explant surpassed the other explants in all parameters under study (necrosis, browning, explant development and callus production).

3. Accumulation of phenolic compounds which resulted from oxidation decreased to the lowermost level when combined treatment (anti-oxidant solution and PVP) was used and in turn all parameters under study are improved.

4. Storing explants in the refrigerator (5°C) for 5 days before culturing succeeded in reducing necrosis, browning and increasing explant development and callus production. However, necrosis and browning increased by using either 1 day or 3 days.

5. Addition 6.0 mg/L 2,4-D to culture medium increased the explant development and callus production, while increasing 2,4-D concentration increased the necrosis and browning.

6. Using 4.0 mg/L BAP maximized callus production and explant development parameters followed by either 2.0 or 6.0 mg/L concentration.

7. Yeast extract surpassed the other organic additives in reducing necrosis while increasing callus maturation, No. of lobes / callus and No. of somatic embryos / callus.

8. Supplementation of the culture medium with 2.0 mg/L 6-benzylaminepurine and 1.0 mg/L indole-3-butyric acid as hormonal balance encouraged the highest callus maturation, number of lobes / callus, and number of somatic embryos/ callus.

CONCLUSION

Culturing of Marjoram shoot tips and subjected to pre-treated with antioxidant solution as cold on MS medium supplemented with PVP and Adenine sulphate improved establishment stage. Also, adding 2.0 mg/L BAP maximized proliferation. In addition, using half MS strength medium supplemented with 4.0 mg/L GA_3 maximized shoot elongation beside culturing the explant in medium contained 2.0 mg/L IBA enhanced rooting. On the other hand, culturing of the apical bud segments of Meadow saffron pretreated with same treatments of marjoram on MS medium supplemented with either 6.0 mg/L 2,4-D or 4.0 mg/L BAP enhanced the highest callus production. Culturing of produced mature callus on the medium contained yeast extract maximized callus development culturing of the developed callus on medium with hormonal balance 2.0 mg/L BAP plus 1.0 mg/L IBA maximized somatic embryos formation.