Pak. J. Bot., 38(2): 385-391, 2006.

EFFECT OF DIVERSE HORMONAL REGIMES ON *IN VITRO* GROWTH OF GRAPE GERMPLASM

GHULAM MUSTAFA SAJID, MUHAMMAD KASHIF ILYAS AND RASHID ANWAR

Institute of Agricultural Biotechnology and Genetic Resources (IABGR), National Agricultural Research Center (NARC), Islamabad, Pakistan
E-mail of corresponding author: mustafasajid2000@yahoo.com

Abstract

Two accessions of grape germplasm were tested on Murashige-Skoog media containing varying levels of BAP either or not containing IAA and NAA for *in vitro* growth parameters. It was observed that shoot number and shoot mass increased when the concentration of BAP was increased in a linear fashion. The shoot length of both the accessions showed a downward trend when the concentration of BAP was increased. The presence of IAA or NAA in the media, however, had a positive effect on the shoot length even in the presence of BAP (0.2 mg/l). It was possible to induce root induction on hormone free media for both the accessions used but the presence of NAA or IAA in the media had a profound effect on root induction of both the accessions. NAA was found to be more effective in root induction than IAA. Increased levels of BAP were found to inhibit root mass accumulation in both the accessions but the degree of inhibition was found to be accession specific.

Introduction

Grapes belong to the *Vitaceae* family, which comprises of about 60 species. The genus *Vitis* is broadly distributed, largely between 25° and 50° N latitude in eastern Asia, Europe, the Middle East, and North America. Additionally, a few species of *Vitis* are found in the tropics-Mexico, Guatemala, the Caribbean, and northern South America. There are over 100 species reported in the literature, 65 of which are thought to be genuine and another 44 are questionable, probably they are interspecific hybrids. However, the principal species from which the cultivated grape has been derived is *Vitis vinifera. V. vinifera* cultivars are widely regarded as having the highest quality of fresh and dried fruit and wine production. In Pakistan, only European grapes are cultivated for human consumption. Over 70% of grapes are grown in Balochistan, while there is some acreage in NWFP. A survey of northern areas of Pakistan and NWFP shows that there is a considerable amount of biodiversity available in the grape germplasm for exploitation in genetic improvement and characterization for profitable cultivation of grapes. As a part of germplasm conservation and collection strategy, a number of different grape accessions have also been recently introduced from Japan and USA for their potential utilization in Pakistan. World's native grape habitat is threatened in all areas of its range. Expanding population and agriculture eliminated large expanses of habitat and political instability and economic decisions have limited both habitat and conservation of critical germplasm collection. Since grape is a vegetatively propagated crop, there is a great threat to the existing biodiversity and to the germplasm collection introduced from other countries. It is, therefore, imperative to find ways to conserve the germplasm of such a valuable fruit species. Conservation of such species is vital for poverty reduction and sustainable productivity.

In vitro conservation is a method of choice for germplasm conservation of those species which do not produce seeds and are vegetatively propagated or whose seeds cannot be used for plant production because of the heterogeneity of the resulting plant population or which have recalcitrant seeds (Sajid *et al*., 2003). This method has been used for storage of grape germplasm in many ways such as storage at low temperature or growth retardation with the help of osmotica compounds, for prolonged conservation (Ishtiaq & Sajid, 2001; Rousseva, 2001) on short term and medium term basis, whereas cryopreservation has been in practice for long term storage of germplasm. In this paper, we report the hormonal effects on two of the grape accessions in our collection, which were acquired from Japan or from our local collection. Research investigations for successful *in vitro* culture establishment and for studies on effects of different hormonal regimes are crucial for establishing *in vitro* gene bank of those species which are vegetatively propagated such as grape. This paper reports the *in vitro* growth responses of two accessions of grape to diverse hormonal regimes and will facilitate expanding the *in vitro* gene bank of other collections of grape collected from diverse ecologies of Pakistan and elsewhere, and presently being maintained at the field gene bank at our institute for national economic growth on sustainable basis.

Materials and Methods

Plant material: The germplasm of grapes was collected from the Northern areas of Pakistan and established at the field gene bank in the Plant Genetic Resources Program of the Institute. This material was used as an explant source for *in vitro* culture establishment and subsequently to study the effects of various hormonal regimes on *in vitro* growth response of grape plants as reported here. The accessions viz., Wild Grape (WG) and Sundar Khani (SK), which were previously established as *in vitro* cultures have been used in this study.

Growth media: In our preliminary studies, we found that Murashige-Skoog (1962) media used at 75% salt levels were more appropriate for grape culture establishment and subsequent multiplication as compared to the full 100% levels of salts. Therefore, in these studies, we used these levels of salts other than FeEDTA, which was used at 100% strength, in combination with different hormonal complements as described below. The media was prepared with 75% MS salts and full strength vitamins and 100mg/l inositol, 30 gm/l sucrose. Five concentrations of BAP (i.e. 0, 0.1, 0.2, 0.3, 0.5, 0.75 mg/l); one concentration of NAA (i.e. 0.3mg/l); one concentration of IAA (i.e. 0.3 mg/l) were tested. The media were gelled with 8g/l agar (Difo). pH of the media was adjusted at 5.8 before dispensing 70ml aliquots of the media in glass jars. The jars were autoclaved at 15 psi for 17 minutes and stored at 20^{o}C till use.

Culture incubation conditions: The explants were taken from already established *in vitro* material in the *in vitro* conservation lab and prepared by aseptically sectioning the plants to obtain single nodes which were cultured in the glass jars and were incubated in the growth room under fluorescent light of 1000 lux and at a temperature of 20^{o}C and a photoperiod of 16 hours daily. The data on number of shoots, shoot length, root mass, shoot mass, number of nodes, and mortality were recorded and analyzed to study the effect of different hormonal regimes on these *in vitro* growth parameters.

Results and Discussion

In a series of experiments, two grape accessions were tested on MS media containing varying levels of BAP either with or without the auxin IAA or NAA. The growth responses are given in Tables 1. The plant cultures have been shown in Fig. 1-A-G.

Shoot length: When BAP was increased from 0 to 0.75 mg/l, it was observed that it had a retarding effect on shoot length of wild grape accession in linear fashion, which decreased from 9.34 cm to 3.1 cm in case the growth media was devoid of IAA or NAA. On the other hand, the presence of IAA or NAA in these media reduced the retarding effect of BAP on shoot length of this accession. IAA was found to have more influence than the NAA on reduction of BAP effects. Thus, both the auxins have a positive effect on shoot elongation of this accession even in the presence of lower levels of BAP. A similar pattern of effect of BAP was observed on the other accession viz., Sundar Khani. When explants of this accession were cultured on the MS media containing levels of BAP ranging from 0 to 0.75 mg/l, the shoot length almost linearly decreased from 9.5 to 3.4 cm in case there was no IAA or NAA in the media. The presence of IAA or NAA in the media promoted shoot length even in the presence of BAP. NAA was found to be more effective in promoting shoot length of this accession. BAP on the other hand has a negative effect of shoot length in both the accessions.

Shoot number: The shoots of Wild grape accession did not proliferate on those media which were either devoid of any hormone or containing low levels of BAP or auxins tested in these studies. The explants grown on these hormone regimes showed shoot elongation only without shoot proliferation in numbers. However, when BAP was increased beyond 0.3mg/l, there was an increase in the number of shoots per explant and a maximum of 2.8 shoots of Wild grape accession were harvested from the media containing 0.75 mg/l. The other accession, Sundar Khani showed mild shoot proliferation on media containing no or low levels of BAP but shoot proliferation was profoundly increased at higher level of BAP tested and 10 shoots were harvested from the media containing 0.5 or 0.75 mg/l BAP. Thus, there was a remarkable difference between the two genotypes in their shoot proliferation response to the increasing levels of BAP. IAA or NAA were found to have no effect on shoot proliferation of either genotype. Tapia and Read (1998) also reported similar findings when they grew two different genotypes on the media containing BAP and NAA. However, they observed severe necrosis and vitrification of cultures when they omitted NAA from the media. Our cultures did not exhibit such phenomenon on any media. This may be attributed to genotypic differences.

Node numbers: There was a strong linear relationship between the number of nodes per shoot and the level of BAP. As the BAP level increased, the number of nodes also increased specially in the higher range of the cytokinin tested. IAA also had a positive effect on number of nodes if the BAP was kept constant in the media. Sundar Khani was found to be more responsive to BAP levels as it yielded more number of nodes than wild grape especially at higher levels of the cytokininn. Kara (1995) also reported similar findings for the tissue cultured grapes while perusing the propagation studies of this species.

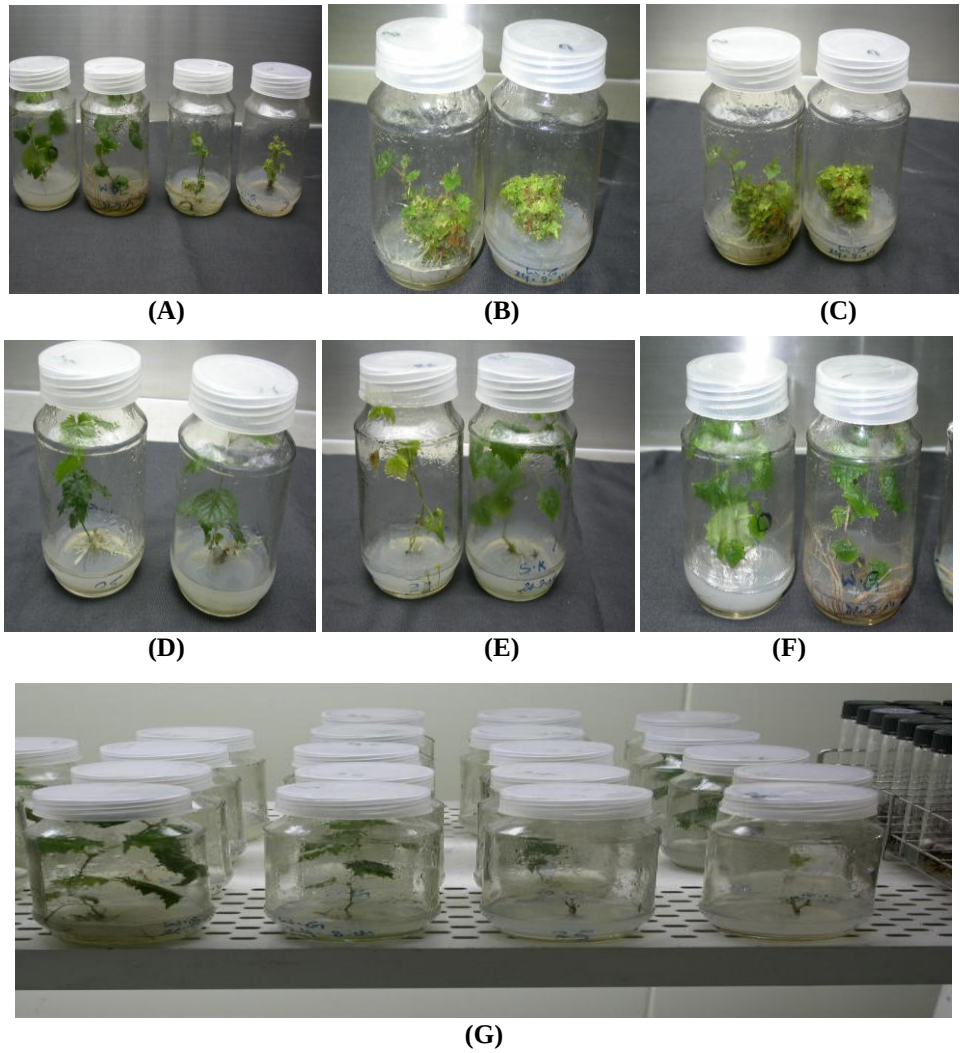

Fig.1. (A) Grape cultures showing different growth stages after establishment *in vitro*.
(B and C) Plant cultures exhibiting profuse shoot growth when grown on media containing BAP (0.75mg/l).

(D-E) Plant cultures showing single shoot growth without shoot multiplication, when grown on media containing no BAP.

(F) Plants exhibiting root growth when grown on media containing NAA.

(G) A battery of plant cultures grown on media containing diverse growth hormonal regimes under incubation in the growth room.

Shoot mass: In the absence of BAP, both the accessions showed larger shoot mass than on the media containing low levels of BAP (less than 0.2mg/l) but at the higher levels of BAP, both the accessions yielded increasingly larger shoot masses as the concentration of BAP increased from 0.3 to 0.75mg/l. Highest shoot mass was obtained when the highest levels of BAP were supplemented in the growth media in case of both the accessions. Between the two accessions, however, Sundar Khani gathered more mass at each of the regime tested. Doroshenko (1996) has reported that very low levels (0.05mg/l) of BAP in the medium have a stimulatory effect on shoot multiplication. The laboratory conditions and other media preparation conditions may vary from lab to lab which may result in differences in optimal conditions for best multiplication protocols. Choi *et al*., (1992) have reported that half strength MS media was more suitable for meristem establishment of grapes but recommended the full strength MS media supplemented with BAP and IAA for maximizing the shoot proliferation. They also used kinetin to study the effect on shoot multiplication and found no special effect. Previously, Saira & Sajid (2005) have reported that BAP affects the shoot mass of sugarcane cultures as its concentration is increased along with the auxin in the media.

Root mass: When wild grape plants were cultured on MS media containing no hormone, they developed rooting but root mass showed a decline as the media contained higher concentration of BAP. Thus, BAP was found to inhibit the root induction. However, IAA and NAA in the media containing BAP (0.2 mg/l) promoted root induction even in the presence of BAP. NAA proved to be better in root induction as compared to IAA in both the accessions. It was observed that Sundar Khani showed lesser root mass on the hormone -free media as compared to wild grape but it too exhibited root inhibition by the increasing levels of BAP except at very high levels of BAP, where it showed some increase in root induction. Gok *et al*., (1997) have reported root induction on hormone free media or the media containing IBA.

Mortality: Culture mortality was found to be influenced by the hormonal regime on which the cultures were grown. It was observed that 100% cultures of each of the accession used in this study, survived on the media containing higher levels of BAP (0.75mg/l). At lower levels of BAP, there was a varying degree of culture mortality although there was no specific trend related to the increasing strength of the cytokinin in the media. Tapia *et al*., (1998) have reported that NAA was essential for culture survival especially when high BAP concentration (2.0 mg/l) were used without NAA. We did not encounter such culture detrimental effects of higher BAP levels in the media as we did not exceed 0.75 mg/l level in our studies. In our previous studies, we reported that culture mortality is completely excluded once the cultures are successfully established as *in vitro* after initial surface disinfection and during the subsequent transfers of cultures, culture contamination can be conveniently eliminated by observing the aseptic conditions (Kashif & Sajid, 2005).

Evaluation of different hormonal regimes for *in vitro* growth responses of the two accessions of grape germplasm under the present study has strengthened our understanding on culture establishment, rooting and shoot proliferation which are vital in maintaining the *in vitro* gene bank. Other hormones such as kinetin, adenine sulfate, thiadizuran and IBA may be included in future studies for growth promotion and growth retardation of grape cultures to strengthen the capacity for *in vitro* gene bank management.

Acknowledgement

The research work for this study was funded by the Agricultural Linkages Program (ALP) which is executed by the Pakistan Agricultural Research Council, Islamabad.

References

Choi, S.Y., J.Y. Oh, J.S. Kim, D.M. Pak, S.B. Lee and D.U. Choi. 1992. Studies on the grape meristem culture *in vitro*. 1. Factors affecting culture establishment and shoot proliferation of Kyoho and S.9110 grape varieties by meristem culture *in vitro*.
Research Reports of the Rural Development Administration, Biotechnology. 34(2)1-9.

Doroshenko, N.P. 1996. Optimization of the conditions for clonal micropropagation in grape vine. *Sel'skokhozyaistvennaya Biologiya*, 5: 76-81.

Gok, S., F. Ergenoglu, A.B. Kuden and F.G.Jr. Dennis. 1997. Propagation of several grape varieties and rootstocks by meristem culture. *Acta Horticulturae*, 441: 245-250.

Ishtiaq, M., G.M. Sajid and R. Anwar. 2001.*In vitro* Conservation of Potato Germplasm. *Pakistan Journal of Biological Sciences*. NARC. Islamabad. 4: 537-538.

Kara, S. 1995. Propagation of grapes by tissue culture. *Ege-Universitesi-Ziraat-Fakultesi-Dergisi*, 32(2): 155-159.

Kashif, M., G.M. Sajid and R. Anwer. 2005. Effect of duration of mercuric chloride treat,ment on culture viability, contamination and mortality of various accessions of grapes. *Sarhad Journal of Agriculture*, 21(1): 25-28.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*, 15: 473-497.

Rousseva, R. 2001. Study of the possibilities of *in vitro* storage of grapevine (*Vitis vinifera* L.). *Rasteniev dni-Nauki*., 38 (7): 374-376.

Saira, P., G.M. Sajid, R. Anwer and H. Rahman. 2005. Hormone dependent growth promotion and growth retardation of sugarcane tissue cultures for germplasm conservation. *Journal of Biological Sciences*, 5(3): 339-346.

Sajid, G.M., S.D. Siddique, M. Ishtiaq, A.U. Haq and A. Rashid. 2003. *In vitro conservation of vegetatively propagated crops: A laboratory manual*. JICA-PARC publication.

Tapia, M.I. and E. Read. 1998. Propagation of grape hybrids by in vitro culture of axillary buds. *Agro. Ciencia*., 14(1): 35-41

(Received for publication 11 May 2005)