Virus Elimination, in vitro response and colonel multiplication Potato (*Solanum tuberosum*)

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Keywords

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Abstract

Meristem culture has become a powerful and successful tool for virus elimination from infected plants and has been successfully applied in potato. The potato (*Solanum tuberosum* L.) tubers for each of three cultivars that grown in pakistan were obtained from the NARC Islamabad. After the thermotherapy, meristems of these potato varieties were cultured for shoot proliferation and root induction in Murishige and skoog (MS) medium supplemented with different types and concentrations of phytohormones. For the initiation of meristems Gibbrelic acid (GA3) with four concentrations (0,1, 2,3, and 4mg) was used. After DAS-ELISA, the virus free in vitro grown potato plantlets were used for massive propagation. The parameters included were number of shoots, no of nodes, no of leaves, plant height and root length. Regarding the rate of initiation the maximum growth was achieved at 1mg/l of GA3. The higher number of nodes and higher number of leaves were produced by GA3 at 2mg/l. For higher frequency of root formation BAP+GA3 was the most effective combination.

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Introduction

Potato (*Solanum tuberosum* L.) is the fourth most cultivated food crop after wheat, rice and maize (Moeinil *et al.,* 2011). It is a native of South America; in sixteenth century Spanish explorers introduced it in Europe and later it became an important food crop of the world (Khoso, 1988). In Pakistan during the year 2008 its production was 2.5 million metric tons (FAO, 2008). virus and viroid like diseases are major diseases in potato seed production and certification. They include Potato leaf Roll virus(PLRV), potato virus A, Potato virus S (PVS), Potato virus M (PVM) (Abu jawadah *et al.,* 2001).The presence of viral diseases are among the major attributes to low yield of potato varieties; the yield reduction may upto 75% caused by the infection of viruses. As such PVX alone may cause a reduction up to 15-30%; PLRV and some strains of PVY may cause reduction up to 50 -70% in seed tuber production(Mellor et al 1987). Meristem tissues are virus free because the vascular bundles are not present in these tissues (kantharaja 2001). Meristem tissue culture is successfully applied in production of virus free potato plants and increase in yield was obtained by the use of these virus free plants (wang *et al.,* 2001). Potato virus free clones with meristem culture were also applied by Ebadi *et al.,* (2007) and Najib *et al.,* (2003). Meristem culture along with thermotherapy has become a powerful tool in Elimination of potato viruses like PVX, PVY and PLRV from infected plants.(Zaman *et al.,* 2001and Ficcoli *et al.,* 2001). In plant diagnostic work the use of ELISA test and molecular techniques have increased in recent years in detection of plant pathogens necessary for potato certification programmes. The ELISA technique has become a standard technique for detection of plant viruses and especially for potato viruses (shojaie *et al.,* 2009). Conventionally, the crop is propagated asexually by tubers. However, this vegetative propagation contaminates tubers by different diseases resulting in poor quality and yields. Alternatively, micropropagation methods are ideal for rapid multiplication of disease free material in masses. Meristem tip culture is an effective method for the production of virus free plants (Badoni and Chauhan 2009) as meristems are virus free. In addition, in vitro methods can be used for conservation, storage and easy distribution of potato germplasm in the form of breeding lines, new varieties and microtubers. Although, there are many reports on potato micropropagation (Yousef *et al.,* 2001; Badoni and Chauhan, 2009; Rahman *et al.,* 2010), It is a well known fact that the regeneration potential of micropropagated plants is genotype dependent (Abe and Futsuhara 1986). Thus, the present investigation was carried out to produce virus free plants by using thermotherapy and meristem culture and to optimize the best combination of growth regulators (GRs) for the multiplication of potato varieties; the most regenerative variety could be then efficiently micropropagated for commercial purposes and molecular studies.

Materials and Methods

Plant material

Field grown potato tubers were obtained from potato programme National Agriculture Research Center Islamabad, Pakistan. The Planting materials Field grown tubers cv symfonia, courage and harmis were treated with 200 ppm solution of Gibberllic acid (GA3) for 20 minutes to break dormancy and incubated in dark conditions at room temperature for 26-28 C° for one week. After sprouting tubers were sown in pots in a thermotherapy chamber for further growth at temperature 34-37 C° for 21 days. The plants

were periodically irrigated with Hogland's nutrient solution. The excised meristems were cultured in MS medium (1962) containing 1mg/l of GA3 on filter paper bridges in test tubes. The cultures were kept 16h photoperiod at temprature 25 C°.

Enzyme Linked Immuno Sorbant Assay (ELISA)

Serological ELISA test was conducted for detection of viruses (PLRV, VPX, PVY, PVA, PVM and PVS) of freshly initiated in vitro stock of all the three varieties after thermotherapy and meristem culture. The leaf samples were collected from in vitro plants and tested by using ELISA from Federal Seed Certification and Registration Department Islamabad, Pakistan. The procedure described by Clark, (1981) was adopted for ELISA.

Multiplication

The meristem derived plants were transferred for multiplication on MS medium containing different concentrations of BAP, GA3 IAA, IBA and kinetin. The effect of these growth regulators on five concentration levels (0, 2, 3, and 4 mg/l) were used to produce virus free plantlets. The parameters studied were, number of shoot, plant let height, higher number of nodes, maximum number of leaves/plant let and root length.

Results and Discussions

Survival percentage of Meristem

The first parameter studied during experiment was survival percentage of meristems of three varieties, symfonia, courage and Harms. A total of 70 meristems were cultured for each variety and highest survival percentage (85.7) was shown by harmis followed by courage (81.4) and symfonia showed least percentage of meristem survived (71.40). Table-1.

ELISA

The initiated plantlets were subjected to sensitive ELISA Technique for detection of incidence of viruses; viz PVX, PVY, PVA, PLRV, PVM and PVS. 21 Plants of each variety were subjected to double antibiotic sandwich enzyme linked immuno sorbant Assay. It is evident from results that all treated plants were tested negative for viruses. The test also reveals that meristem culture along with thermotherapy is successful technique for elimination of viruses. The test was also in conformity with the findings of Truskinov and Rogozina (1997) and Rancovic et al., (1997). The results were also similar to Ghafoor et al. (2003) who used meristem culture along with thermotherapy to produce virus free plants. Table-2.

Number of Shoots

The highest number of shoots during multiplication were observed for variety symfonia (3.40) followed by Harmis (3.25) and courage showed (3.00) number of shoots (3.75) were produced by hormone (BAP + IBA) followed by (BAP + GA3) 3.50. The significant interaction was recorded between hormone and concentration which range from 1.00 (Control) to 3.75 (BAP + IBA, 2 mg/l). The interaction between variety and concentration was also found significant from 1.00 (Control) to 3.40 by symfonia at 3 mg/l. The interaction between variety concentration and Hormone was also significant. The maximum number of shoots (3.75) is produced by variety courage and hormone (BAP + IBA) at 2 mg/l and some number of shoots (3.75) was produced by variety symfonia) 3.75) at 2 mg/l by BAP +IBA.

Number of Nodes

The data collected regarding the number of nodes for all three varieties showed that highest number of nodes (3.90) were produced by variety courage. The hormones used for in vitro multiplication of varieties showed significant difference among them. The highest number of nodes were produced by GA3 (4.20) followed by kinetin (3.967) and minimum number of nodes (3.65) were produced by BAP + GA3. The effect of

concentration level on number of nodes (4.59) was produced by 2 mg/l. The interaction of variety and concentration was non-significant. A significant interaction between variety and hormone was recorded which ranged from 4.50 by variety courage and hormone GA3 to 2.35 by variety harmis and hormone BAP + IBA.

Number of Leaves

The data collected regarding the varieties showed that highest average number of leaves 4.63, was produced by the variety symfonia followed by courage (4.41) and minimum number of leaves (4.10) was produced by variety harmis. The means of all three varieties regarding the number of leaves were significantly different from each other.

The highest number of leaves (4.78) was produced by the hormone GA3 followed by kinetin (4.53) and lowest number of leaves (3.93) were produced by BAP + IBA. The means of all the five hormones were significantly different from each other at 5% level of significance. Regarding the concentration the data showed that maximum number of leaves (5.05) was produced by the concentration 2mg/l followed by 3mg/l (4.81). The minimum number of leaves was produced by controlled conditions. The means of all the concentrations was significant from each other at 5 % level of significance.

The interaction of hormone and concentration was significant. The maximum number of leaves (5.667) was produced by GA3 at 2mg/l followed by kinetin and Kinetin +GA3 (5.25) at 4 and 3 mg/l. The lowest number leaves were produced by controlled conditions (3.08). All the means collected for number of leaves were significantly different from each other at 5% level of significance.The interaction of all three factors i e variety, hormone and concentration were significantly different from each other.

Plant Height

The maximum plant height (5.89cm) was produced by variety Symfonia followed variety Courage (4.78) and variety Harmis produce 4.76) plant height. The means all three varieties Symfonia, Courage and Harmis were significantly different from each other at 5 % level of significance. Regarding the hormones the maximum plant height (6.59cm) was produced by the hormone GA3 followed by (5.63) Kinetin and 5.30 by BAP+ GA3. The minimum plant height (3.57) was produced by BAP+ IBA. The means recorded for plant height were significantly from each other at 5% level of significance.

The maximum plant height (5.80) was produced at 4mg/l followed by (5.76) at 2mg/l and (5.60) at 3mg/l.The plant height (3.41) was produced by 0mg/l. The means regarding the concentrations were significantly different from each other at 5% level of significance. The interaction of variety, concentration and hormone was non-significant. Concentration and variety, hormone and concentration were also non-significant.

Root Length

The maximum root length (1.406 cm) was produced by variety Harmis followed by Symfonia (1.404 cm) and minimum root length (1.339 cm) was produced by variety courage. The means recorded for all three varieties were non significant from each other

The data regarding harmones showed that maximum root length (1.78 cm) was produced by BAP + GA3 followed by GA3 (1.35 cm). The minimum root length (1.12 cm) was produced by Kinetin. The means recorded regarding the hormnes showed significant difference from each other at 5 % level of significance. The data regarding the concentration showed that maximum root length (1.94 cm) was observed at 2 mg/l, followed by (1.52 cm) at 4 mg/l. The minimum root length (0.50 cm) was observed at controlled conditions. The means recorded

for root length were significantly different from each other at 5 % level of significance.

Potato are seemed to be infected by many viruses. The purpose of this study is to demonstrate the serological test of the plants after the thermotherapy and meristem culture. The correct diagnosis of a disease is prerequisite for its control, the more rapidly the causal organism is identified sooner it will be controlled. Meristems of three varieties namely Symfonia, Courage and Harmis were established on MS medium in order to produce virus free plants. The meristem derived plants were transferred on MS medium containing different concentrations of BAP, GA3, IAA, IBA and kinetin. The survival percentage of meristems was studied and highest %age (85.7) was shown by harmis. The initiated plantlets were subjected to ELISA test for detection of viruses. The verities used in the study were symfonia, courage and harmis. These three verities were compared for multiplication. The variety symfonia showed highest number of shoots (3.40) followed by harmis (3.25) and courage produced (3.00). Among different media combinations used for invitro multiplication of potato varieties the best media combination was (BAP+IBA) followed by (BAP+GA3). The similar results were achieved by Nagibe *et al.,* (2003). Among different media combinations used by Nagib *et al.,* (2003). The maximum number of shoots (4.36) was achieved by (BAP+IBA) at 1mg/l. Yasmin *et al.,* (2003) obtained maximum number of shoots by using (NAA+BAP) at 2 mg/l. and this media combination took less time for regeneration. The similar results were also obtain by khatoon *et al.,* (2003). The nodal segments of diamante cultivar of potato form invitro grown plantlets were cultured for callus induction and regeneration the maximum number of shoots (8.60) were achieved in 5.0 mg/l BAP+0.1mg/l IBA. Hussain *et al.,* (2005) reported that BAP played important in the regeneration .At lower Concentration shoot number were (0.8) but it increased gradually with increase in BAP to 5.00 number of shoots collectively from all the varieties. Similarly result were also reported by Sarkar and Mustafa (2002) that the BAP showed better response in term of shoot per explant, shoot length number of nodes and leaves in potato varieties. Among the other media combinations used for number of shoots, the BAP +GA3 also showed good result but increase concentration of BAP +GA3 the number of shoot become decreased. Regarding the number of nodes/plantlet the highest number of nodes were produced by GA3 at 2mg/l. Rabbani *et al.,* (2001) reported that the number of nodes were not significantly affected by the any concentration of GA3. Mommani *et al.,* (2000) reported that 0.3 mg/l GA3 the nodal segments gave highest shoot length highest shoot number and nodal number of any explant and media combinations. The longest internodal length of all the explants was on MS supplemented with (0.3mg/l) GA3 while the addition of BA to this Combination decreased the inter nodal length. The maximum number of leaves were produced by variety symfonia. The highest number of leaves were produced by GA3 (4.78). The maximum number of leaves (5.667) were produced by GA3 at 2mg/l. Ghafoor et al (2003) observed highest number of leave (6.143) by lAA. Zaman et al (2001) also reported that lAA produce highest number of leaves in the study GA3 among all the media Combinations was the best for no of leaves. The maximum plant height (5.89cm) was produced by variety symfonia. Regarding the growth regulators the GA3 produced highest plant height (6.59cm) followed by Kinetin .The best Concentration was L1 mg/l. The maximum plant height (5.89 cm) was produced by variety symfonia. Regarding the growth regulations, the GA3 produced highest plant height (6.59 cm) followed by Kinetin.

The best concentration was 4 mg/l. It means higher the concentration of growth regulators higher will be the plant height. Nagib *et al.*, (2003) reported that highest shot length was achieved by IBA at 1 mg/l but in this study the IBA was used in combination with BAP and the shoot length produced was significantly low from other media combinations. Rabbani et al. (2001) reported that maximum shoot length (8.96 cm) was obtained when 4 mg/l GA3 was applied. Mommani *et al.*, (2000) also reported that maximum shoot length was achieved by GA3 at 0.3 mg/l. The maximum root length (1.78 cm) was produced by BAP+GA3 followed by GA3 (1.35 cm). The maximum root length (2.817 cm) was observed by BAP+GA3 at 2mg/l. Ghafoor *et al.*, (2003) reported that highest root length (4.429 cm) was produced by IAA at concentration level of 0.25 mg/l followed by NAA and IAA with similar value (4.28 cm) at the same concentration. Zaman *et al.*, (2003) reported highest root length (4.2 cm) was recorded on IAA at 1 mg/l followed by NAA at 1 mg/l (3.8 cm). In the present study it is reported that maximum root length was produced by BAP +GA3 at 2 mg/l.

In the present study it was concluded that thermotherapy along with meristem culture was effective for virus eradication. Regarding the media composition, the BAP + IBA at 2 mg/l was best for maximum shoot proliferation. The media composition BAP+ GA3 was also effective for maximum shoot proliferation. Regarding the number of nodes, number of leaves and plant height, the GA3 was the most effective media combination. Higher the concentration of GA3, higher will be number of leaves and higher will be the plant height. The best root length was produced by BAP+GA3 at 2 mg/l. the varieties showed equal response. There was no highly significantly difference among the varieties. Overall it was concluded that BAP and GA3 were the best media regimes.

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Table ELISA showing results of meristems for virus detection

Varieties	Meristems cultured	Survived	Contaminated	%age survived
Symfonia	70	50	20	71.40
Courage	70	57	13	81.4
Harmis	70	60	10	85.7

Variety	PVX	PVS	PVA	PVM	PLRV	PVY
Symfonia	-	-	-	-	-	-
Courage	-	-	-	-	-	-
Harmis	-	-	-	-	-	-

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Table 1. Mean number of shoots per plantlet observed at various levels of growth regulators on potato cultivars, Symfonia, Courage and Harmis.

Varieties (V)	**Concentration (C)**					Mean
	0 mg/l	1 mg/l	2 mg/l	3 mg/l	4 mg/l	
V1 (Symfonia)	1.00 i	2.15 f	3.00 bc	3.40 a	2.75 cd	2.46 a*
V2 (Courage)	1.00 i	2.25 ef	3.00 bc	3.05 bc	2.55 de	2.43
V3 (Harmis)	1.00 i	2.35 ef	3.20 ab	3.25 ab	2.35 ef	2.43
Hormones			**(HxC)**			
H1 (GA_3)	1.00 i	2.00 h	3.83 def	3.33 b	3.36 bcd	2.46 a*
H2 (BAP+GA_3)	1.00 i	2.50 fg	3.50 ab	3.17 bcd	2.42 g	2.52
H3 (BAP+IBA)	1.00 i	2.00 h	3.75 a	2.91 cde	2.00 h	2.33
H4 (Kinetine)	1.00 i	2.33 gh	2.50 fg	3.33 b	2.50 fg	2.33
H5 (Kin+GA_3)	1.00 i	2.41 g	3.25 bc	3.41 ab	2.66 efg	2.55
Interaction			**(VxHxC)**			
V1 x H1	1.00 i	1.50 h	2.50 def	3.50 ab	3.50 ab	2.40 a*
V1 x H2	1.00 i	2.50 def	3.25 abc	3.25 abc	3.25 abc	2.65
V1 x H3	1.00 i	1.75 gh	3.75 a	3.25 abc	2.00 fgh	2.35
V1 x H4	1.00 i	2.25 efg	2.25 efg	3.25 abc	2.50 def	2.25
V1 x H5	1.00 i	2.75 cde	3.25 abc	3.75 a	2.50 def	2.65
V2 x H1	1.00 i	2.25 efg	3.75 a	3.25 abc	2.75 cde	2.60
V2 x H2	1.00 i	2.25 efg	3.50 ab	2.50 def	2.25 efg	2.30
V2 x H3	1.00 i	2.25 efg	3.75 a	2.75 cde	2.25 efg	2.40
V2 x H4	1.00 i	2.25 efg	2.50 def	3.25 abc	2.50 def	2.30
V2 x H5	1.00 i	2.25 efg	3.00 bcd	3.50 ab	3.00 bcd	2.55
V3 x H1	1.00 i	2.75 cde	3.75 a	3.75 a	1.75 gh	2.40
V3 x H2	1.00 i	2.00 fgh	3.75 a	2.75 cde	1.75 gh	2.60
V3 x H3	1.00 i	2.50 def	2.75 cde	3.50 ab	2.50 def	2.25
V3 x H4	1.00 i	2.25 efg	3.50 ab	3.00 bcd	2.50 def	2.45
V3 x H5	1.00 i	2.25 efg	3.00 bcd	2.50 ab	3.00 bcd	2.45
Mean	**1.00***	**2.25**	**3.17**	**3.23**	**2.55**	

Any two means carrying the same letter(s) are not significantly different at p=0.05 by LSD test.

Table 2. Mean number of nodes per plantlet observed at various levels of growth regulators on potato cultivars, Symfonia, Courage and Harmis

Varieties (V)	**Concentration (C)**					Mean
	0 mg/l	1 mg/l	2 mg/l	3 mg/l	4 mg/l	
V1 (Symfonia)	2.25 a	4.10 a	4.60	4.40	3.85	3.85 a
V2 (Courage)	2.25 a	4.00 a	4.90	4.80	3.50	3.90 a
V3 (Harmis)	2.00 a	3.65 b	4.20	4.30	3.55	3.54 b
Hormones			**(HxC)**			
H1 (GA_3)	2.16 k	4.25 defg	5.67 a	4.59 bcde	4.33 cdef	4.20 a
H2 (BAP+GA_3)	2.16 k	3.75 ghi	4.58 bcde	4.91 b	2.83 j	3.65 c
H3 (BAP+IBA)	2.16 k	2.83 j	4.16 efg	3.50 hi	3.25 ij	3.18 d
H4 (Kinetine)	2.16 k	4.41 bcde	4.42 bcde	4.83 bc	4.00 fgh	3.97 b
H5 (Kin+GA_3)	2.16 k	4.33 cdef	4.08 efg	4.75 bcd	3.75 ghi	3.82 bc
Interaction			**(VxHxC)**			
V1 x H1	2.25 ijk	4.00 def	5.00 bc	4.00 def	4.50 bcde	3.95 bcd
V1 x H2	2.25 ijk	3.75 efg	5.00 bc	5.00 bc	3.50 fgh	3.90 bcde
V1 x H3	2.25 ijk	3.75 efg	4.25 cdef	3.75 efg	3.50 fgh	3.50 ef
V1 x H4	2.25 ijk	4.75 bcd	4.75 bcd	5.25 b	4.25 cdef	4.25 ab
V1 x H5	2.25 ijk	4.25 cdef	4.00 def	4.25 cdef	3.50 fgh	3.65 cde
V2 x H1	2.25 ijk	5.00 bc	6.75 a	5.00 bc	3.50 fgh	4.50 a
V2 x H2	2.25 ijk	4.00 def	5.00 bc	5.25 bc	3.00 ghi	3.90 bcd
V2 x H3	2.25 ijk	4.50 bcde	5.75 bc	5.00 bc	3.75 efg	3.10 fg
V2 x H4	2.25 ijk	4.75 bcde	4.00 def	5.00 bc	3.75 efg	4.05 bc
V2 x H5	2.25 ijk	4.00 def	5.00 bc	5.25 bc	3.00 ghi	3.95 bcd
V3 x H1	2.00 jk	3.75 efg	5.25 b	5.75 bcd	5.00 bc	4.12 ab
V3 x H2	2.00 jk	3.50 fgh	3.75 efg	4.50 bcd	2.50 jk	3.15 fg
V3 x H3	2.00 jk	3.00 ghi	4.00 def	3.00 ghi	2.75 ij	2.95 g
V3 x H4	2.00 jk	4.00 def	3.75 efg	4.25 cdef	4.00 def	3.60 de
V3 x H5	2.00 jk	4.00 def	4.25 cdef	5.00 bc	4.00 def	3.85 bcde
Mean	**2.17 d**	**3.92 b**	**4.59 a**	**4.52 a**	**3.64 c**	

Any two means carrying the same letter(s) are not significantly different at p=0.05 by LSD test.

Table 3. Mean number of leaves per plantlet observed at various levels of growth regulators on potato cultivars, Symfonia, Courage and Harmis

Varieties (V)	**Concentration (C)**					Mean
	0 mg/l	1 mg/l	2 mg/l	3 mg/l	4 mg/l	
V1 (Symfonia)	3.50	4.75	5.10	4.70	4.70	4.63 a
V2 (Courage)	3.25	4.60	4.70	4.35	4.35	4.41 b
V3 (Harmis)	2.50	4.20	4.65	4.25	4.25	4.10 c
Hormones			**(HxC)**			
H1 (GA_3)	3.12 g	5.00 bc	5.66 a	5.00 bc	5.16 ab	4.78 a
H2 (BAP+GA_3)	3.12 g	4.58 cde	4.83 bcd	4.25 f	3.50 g	4.05 c
H3 (BAP+IBA)	3.12 g	4.08 f	4.92 bcd	4.33 ef	3.25 g	3.93 c
H4 (Kinetine)	3.12 g	4.50 cdef	4.91 bcd	5.25 ab	5.25 ab	4.60 ab
H5 (Kin+GA_3)	3.12 g	4.41 be	4.91 bcd	5.25 ab	5.00 bc	4.53 b
Interaction			**(VxHxC)**			
V1 x H1	2.25 ijk	4.00 def	5.00 bc	4.00 def	4.50 bcde	4.75 a*
V1 x H2	2.25 ijk	3.75 efg	5.00 bc	5.00 bc	3.50 fgh	4.30
V1 x H3	2.25 ijk	3.75 efg	4.25 cdef	3.75 efg	3.50 fgh	4.20
V1 x H4	2.25 ijk	4.75 bcd	4.75 bcd	5.25 b	4.25 cdef	5.05
V1 x H5	2.25 ijk	4.25 cdef	4.00 def	4.25 cdef	3.50 fgh	4.85
V2 x H1	2.25 ijk	5.00 bc	6.75 a	5.00 bc	3.50 fgh	4.95
V2 x H2	2.25 ijk	4.00 def	5.00 bc	5.25 b	3.00 ghi	4.20
V2 x H3	2.25 ijk	2.75 hij	4.25 cdef	3.75 efg	3.50 fgh	4.05
V2 x H4	2.25 ijk	4.50 bcde	4.75 bcd	5.00 bc	3.75 efg	4.40
V2 x H5	2.25 ijk	4.75 bcd	4.00 def	5.00 bc	3.75 efg	4.45
V3 x H1	2.25 ijk	3.75 efg	5.25 b	4.75 bcd	5.00 bc	4.65
V3 x H2	2.00 jk	3.50 fgh	3.75 efg	4.50 bcde	4.50 bcde	3.65
V3 x H3	2.00 jk	3.00 ghi	4.00 def	3.00 ghi	2.75 hij	3.55
V3 x H4	2.00 jk	4.00 def	3.75 efg	4.25 cdef	4.00 def	4.35
V3 x H5	2.00 jk	4.00 def	4.25 cdef	5.00 bc	4.00 def	4.30
Mean	**3.08 d**	**4.51 b**	**5.05 a**	**4.81 b**	**4.43 c**	

Any two means carrying the same letter(s) are not significantly different at p=0.05 by LSD test.

Table 4. Mean number of plant height per plantlet observed at various levels of growth regulators on potato cultivars, Symfonia, Courage and Harmis

Varieties (V)	**Concentration (C)**					Mean
	0 mg/l	1 mg/l	2 mg/l	3 mg/l	4 mg/l	
V1 (Symfonia)	3.92	5.50	6.23	5.93	7.88	5.89 a
V2 (Courage)	3.05	4.97	5.73	5.46	4.68	4.78 b
V3 (Harmis)	3.33	4.88	5.33	5.42	4.85	4.76 b
Hormones			**(HxC)**			
H1 (GA_3)	3.44	5.85	6.93	5.58	5.15	6.59 a
H2 (BAP+GA_3)	3.42	5.38	6.20	4.85	4.55	4.88 b
H3 (BAP+IBA)	3.44	3.50	4.05	3.80	4.92	3.57 c
H4 (Kinetine)	3.44	5.53	5.96	6.37	5.50	5.36 b
H5 (Kin+GA_3)	3.44	5.5 7	5.65	6.41	5.43	5.30 b
Interaction			**(VxHxC)**			
V1 x H1	3.92	5.97	7.00	6.72	9.37	8.60 a*
V1 x H2	3.92	6.12	7.40	5.35	4.45	5.45
V1 x H3	3.92	4.02	4.60	4.05	4.82	4.08
sV1 x H4	3.92	5.87	6.05	6.59	6.10	5.78
V1 x H5	3.92	5.50	6.10	6.60	5.65	5.55
V2 x H1	3.05	5.17	7.95	6.55	5.45	5.63
V2 x H2	3.05	5.73	5.50	4.60	4.62	4.63
V2 x H3	3.05	3.22	4.10	3.85	3.05	3.45
V2 x H4	3.05	5.57	5.97	5.85	5.15	5.12
V2 x H5	3.05	5.50	5.12	6.74	5.15	5.06
V3 x H1	3.35	6.40	5.85	6.47	5.65	5.54
V3 x H2	3.35	4.65	5.72	4.62	5.57	4.58
V3 x H3	3.35	4.50	4.45	4.50	4.30	3.22
V3 x H4	3.35	5.15	5.87	6.32	5.25	5.19
V3 x H5	3.35	5.72	5.75	6.17	5.50	5.30
Mean	**3.44 c**	**5.11 ab**	**5.76 a**	**5.61 a**	**5.81 a**	

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Table 5. Mean number of root length per plantlet observed at various levels of growth regulators on potato cultivars, Symfonia, Courage and Harmis

Varieties (V)	**Concentration (C)**					Mean
	0 mg/l	1 mg/l	2 mg/l	3 mg/l	4 mg/l	
V1 (Symfonia)	0.57 f	1.35 c	1.95 a	1.45 cd	1.50 c	1.40 a
V2 (Courage)	0.57 f	1.24 c	1.81 b	1.51 c	1.44 cd	1.33 a
V3 (Harmis)	0.57 f	1.37 de	2.07 a	1.44 cd	1.52 c	1.40 a
Hormones			**(HxC)**			
H1 (GA_3)	0.59 j	1.47 fg	1.63 def	1.56 ef	1.50 f	1.35 b
H2 (BAP+GA_3)	0.59 j	1.78 cd	2.81 a	1.83 bc	1.85 bc	1.78 a
H3 (BAP+IBA)	0.59 j	1.53 f	1.72 cd	1.49 f	1.48 f	1.36 b
H4 (Kinetine)	0.59 j	1.06 i	1.55 ef	1.13 hi	1.29 gh	1.12 c
H5 (Kin+GA_3)	0.59 j	1.05 i	2.01 b	1.25 h	1.50 f	1.28 b
Interaction			**(VxHxC)**			
V1 x H1	0.57 w	1.27 n-s	1.15 q-t	1.52 i-o	1.35 m-r	1.75 h
V1 x H2	0.57 w	1.95 c-g	3.15 a	1.95 c-g	1.90 c-h	1.90 a
V1 x H3	0.57 w	2.00 c-f	1.87 c-h	1.37 l-r	1.40 k-r	1.44 cd
V1 x H4	0.57 w	1.27 n-s	1.70 f-k	1.00 s-u	1.27 n-s	1.65 h
V1 x H5	0.57 w	1.17 q-t	1.90 c-h	1.40 k-r	1.60 h-m	1.33 d-g
V2 x H1	0.57 w	1.37 l-r	2.00 c-f	1.45 j-q	1.45 j-q	1.37 df
V2 x H2	0.57 w	1.75 d-j	2.37 b	2.10 bc	2.05 cd	1.77 ab
V2 x H3	0.57 w	1.22 o-s	1.40 k-r	1.55 i-n	1.50 i-p	1.25 fgh
V2 x H4	0.57 w	0.77 u-w	1.27 n-s	1.20 p-t	1.25 n-s	1.01 i
V2 x H5	0.57 w	1.01 r-t	2.25 cde	1.25 n-s	1.50 i-p	1.29 efgh
V3 x H1	0.62 vw	1.78 d-i	1.75 d-j	1.72 e-j	1.70 f-k	1.51 c
V3 x H2	0.62 vw	1.65 g-n	2.92 a	1.60 h-m	1.60 h-m	1.68 b
V3 x H3	0.62 vw	1.34 l-r	1.90 c-h	1.55 i-n	1.55 i-n	1.40 cde
V3 x H4	0.62 vw	1.15 q-t	1.67 g-l	1.20 p-t	1.35 m-r	1.20 gh
V3 x H5	0.62 vw	0.90 t-v	2.12 bc	1.12 rst	1.40 k-r	1.23 fgh
Mean	**0.59 d**	**1.38 c**	**1.94 a**	**1.46 b**	**1.52 b**	

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