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Assessment of "in vitro" propagation potential for some gloxinia (*Sinningia speciosa*) genotypes

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Abstract: Gloxinia (*Sinningia speciosa* Hierm) belongs to the family Gesneriaceae and is a commercially important ornamental plant. Gloxinia produces single or double flowers that are available in a variety of colors. This plant can be traditionally propagated by leaf, stem, rhizome, seed, and crown cuttings from a mature plant after blooming. The biological material was composed of five genotypes of Gloxinia (*Sinningia speciosa*): Kaiser Wilhelm; Kaiser Friedrich; Mont Blanc; Prince Albert; hybrid MA. The aim of this study was to investigate the in vitro regeneration potential of different gloxinia genotypes on different hormonal balances, in order to further increase the efficiency of their multiplication rate. The hormonal balance has the highest contribution on the variability of this trait (85.73%), followed by the genotype (5,51 %), while the culture duration had a lower contribution of 2.24 %. Kaiser Friedrich variety recorded average values of shoots number/explant significantly higher to other genotypes. The balance BA4+ANA0.1 proved to be the most effective for the initiation and formation of shoots, thus resulting statistically assured increases of shoots number/explant compared to the other studied hormonal combinations.

Key words

gloxinia, in vitro, propagation potential

Gloxinia (*Sinningia speciosa* Hierm) belongs to the family Gesneriaceae and is a commercially important ornamental plant. Gloxinia produces single or double flowers that are available in a variety of colors. This plant can be traditionally propagated by leaf, stem, rhizome, seed, and crown cuttings from a mature plant after blooming [6]. However, it is difficult to obtain an abundance of healthy progeny, either by seed, due to self-sterility [3], or by tubers [10].

Plants show totipotency or the ability to produce a clone from cells in leaves, stems, roots, etc. Plant proliferation by this mechanism is termed plant regeneration [5; 11]. Whole plants can regenerate from excised plant parts by a number of pathways [2]. The Gloxinia plant developed through tissue culture remains true to type, inexpensive and disease free [1; 7]. Although previous studies have developed regeneration systems of *S. speciosa*, there are several limitations, such as that the regeneration efficiency has yet to exceed 91.5%; the regeneration cycle takes over 2 mo; and the procedures use different kinds of medium [10; 12; 3; 9].

The aim of this study was to investigate the in vitro regeneration potential of different gloxinia genotypes on different hormonal balances, in order to further increase the efficiency of their multiplication rate.

Material and Method

The biological material was composed of five genotypes of Gloxinia (*Sinningia speciosa*): Kaiser Wilhelm; Kaiser Friedrich; Mont Blanc; Prince Albert; hybrid MA. From the mother plants that were grown in vivo, mature leaf tissue was collected, thus obtaining explants of the same size. From each variety plant tissue was collected for 50 explants at each hormonal balance.

The sterilized explants were placed into round Petri dishes containing Murashige and Skoog (MS: Murashige and Skoog 1962) basal medium supplemented with phytohormones in six hormonal balances, to favor the direct somatic embryogenesis: BA 2 mg/l; BA 4mg/l; BA 2 mg/l +ANA 0.1 mg/l; BA 4 mg/l +ANA 0.1 mg/l; BA 2 mg/l +GA3 0,5 mg/l; BA 4 mg/l +GA3 0,5 mg/l. The Petri dishes were placed under cool white fluorescent light with a photoperiod of 16-h light and 8-h dark at 24±1ºC for the induction of shoots.

Data were collected at 2 weeks (when the first shoots have emerged), at 4 weeks (when the shoots were differentiated) and at 6 weeks (when the shoots were mature, good for transplanted on rooting medium).

In order to initiate the "in vitro" cultures, a three factors experiment was organized on the type 5 x 6 x 3, after a completely randomized design, having as factors: the genotype, the hormonal balance and the culture duration. To determine the significance of differences between the various studied factors the statistical analysis was done by ANOVA and t test [4].

Results and Discussions

All three factors have a significant and real influence on shoots formation (Table 1). The hormonal balance has the highest contribution on the variability of this trait (85.73%), followed by the genotype (5,51 %), while the culture duration had a lower contribution of 2.24 %. Also the combined effects of the factors have significantly influenced the shoots formation. The genotype x hormonal balance (3.06 %) and genotype x culture duration (1.53 %) interactions, showed the highest contribution to the variation of shoots number/explant. The triple interaction of the factors had the lowest influence, but statistically assured on this trait.

Table 1

Analysis of variance for the effect of genotype, hormone balance and duration of in vitro culture on gloxinia shoots formation

Source of variation	SS	DF	MS	F Test
Total	8179.87	899		
Genotype	221.41	4	55.35	18.45**
Hormonal balance	4305.76	5	861.15	287.05**
Culture duration	44.95	2	22.48	7.49**
Genotype x Balance	615.47	20	30.77	10.26**
Genotype x Duration	122.57	8	15.32	5.11**
Balance x Duration	112.17	10	11.22	3.74**
Genotype x Balance x Duration	327.54	40	8.19	2.73**
Erorr	2430.00	810	3.00	

Taking into account the unilaterally effect of the genotype (Table 2) it is observed that generally at the level of whole experiment there are differences statistically assured between themselves, in terms of shoots formation. Thus, there is amplitude of 1.72 on a background of a medium interpopulation variability (12.04 %), with the limits from 4.96 for Mont Blanc to 6.68 for Kaiser Friedrich variety.

At the level of whole experience Kaiser Friedrich variety recorded average values of shoots number/explant significantly higher to other genotypes with increases ranging from 9.50% compared to MA hybrid and 25.75% to Mont Blanc variety. Higher values of shoots formation (over 6) and also statistically assured differences of 0.56 to 1.15 were also carried out by the MA hybrid, while the number of shoots formed at Mont Blanc variety was significantly lower than the rest of the genotypes with the exception of Prince Albert.

Table 2

The effect of genotype on shoots number/explant in gloxinia

Genotypes	Shoots number		Relative values (%)	Difference/ Significance
Kaiser Friedrich - Kaiser Wilhelm	6.68	5.55	120.36	1.13***
Mont Blanc - Kaiser Wilhelm	4.96	5.55	89.37	-0.59^{00}
Prince Albert - Kaiser Wilhelm	5.26	5.55	94.77	-0.29
Hibrid MA - Kaiser Wilhelm	6.11	5.55	110.09	0.56**
Mont Blanc - Kaiser Friedrich	4.96	6.68	74.25	-1.72^{000}
Prince Albert - Kaiser Friedrich	5.26	6.68	78.74	-1.42^{000}
Hibrid MA - Kaiser Friedrich	6.11	6.68	91.47	-0.57^{00}
Prince Albert - Mont Blanc	5.26	4.96	106.05	0.30
Hibrid MA - Mont Blanc	6.11	4.96	123.19	1.15***
Hibrid MA – Prince Albert	6.11	5.26	116.16	0.85***

$LSD_{5\%}=0.36$ $LSD_{1\%}=0.45$ $LSD_{0.1\%}=0.60$

Regarding the effect of hormonal balance on shoots number/explant (Table 3) the highest value of 8.56 was recorded when using the combination of BA4+ANA0,1, while the lowest value of 1.56 was

found in the medium with the hormonal balance BA2+GA3 0,5, resulting an amplitude of 5.10. As such, the balance BA4+ANA0,1 proved to be the most effective for the initiation and formation of shoots, thus resulting statistically assured increases of shoots number/explant compared to the other studied hormonal combinations, with relative values ranging from 11% compared to BA2+ANA0,1 and 252 % than BA2+GA3 0,5.

Increasing the concentration of benzylaminopurine from 2 to 4 mg/l caused a very significant increase of shoots number with 10.5%, while the supplementation of medium based on BA with ANA 0,1 mg/l has generated an increase by 15-16% of the value of this trait. The use of gibberellic acid in a dose of 0.5 mg/l has had a negative effect on the formation of shoots leading to a significant reduction with 66-76% of shoots number/explant, compared to the medium based on BA. Changing the concentration of benzylaminopurine in the mixtures with GA3 caused a very significant increase about 55% of this trait.

Table 3

The effect of hormone balance on shoots number/explant in gloxinia

Hormonal balance	Shoots number		Relative values (%)	Difference/ Significance
BA4 – BA2	7.36	6.66	110.51	0.70***
BA2+ANA0.1 – BA2	7.68	6.66	115.32	1.02***
BA4+NAA0.1 – BA2	8.56	6.66	128.53	1.90***
BA2+GA3 0.5 – BA2	1.56	6.66	23.42	-5.10^{000}
BA4+ GA3 0.5 – BA2	2.43	6.66	36.49	-4.23^{000}
BA2+ANA0.1 – BA4	7.68	7.36	104.35	0.32
BA4+ANA0.1 – BA4	8.56	7.36	116.30	1.20***
BA2+GA3 0.5 – BA4	1.56	7.36	21.20	-5.80^{000}
BA4+ GA3 0.5 – BA4	2.43	7.36	33.02	-4.93^{000}
BA4+ANA0.1 – BA2+ANA0.1	8.56	7.68	111.46	0.88***
BA2+GA3 0.5 – BA2+ANA0.1	1.56	7.68	20.31	-6.12^{000}
BA4+ GA3 0.5 – BA2+ANA0.1	2.43	7.68	31.64	-5.25^{000}
BA2+GA3 0.5 – BA4+ANA0.1	1.56	8.56	18.22	-7.00^{000}
BA4+ GA3 0.5 – BA4+ANA0.1	2.43	8.56	28.39	-6.13^{000}
BA4+ GA3 0.5 – BA2+ GA3 0.5	2.43	1.56	155.77	0.87***

$LSD_{5\%}$=0.39 $LSD_{1\%}$=0.51 $LSD_{0.1\%}$=0.66

Regarding the effect of culture duration on the formation of shoots for this species (Table 4), it is noted that average values ranging from 5.49 in two weeks to 5.91 for six weeks were recorded, due to amplitude of 0.42 associated with reduced variability (3.69%). During the period between the second and sixth week the shoots formation increased significantly with 7.6%. The extension of in vitro culture from two to four weeks has had a statistically uninsured effect leading to an increase of shoots number/explant with 4.4%, or 3.1% in the case of extended from four to six weeks.

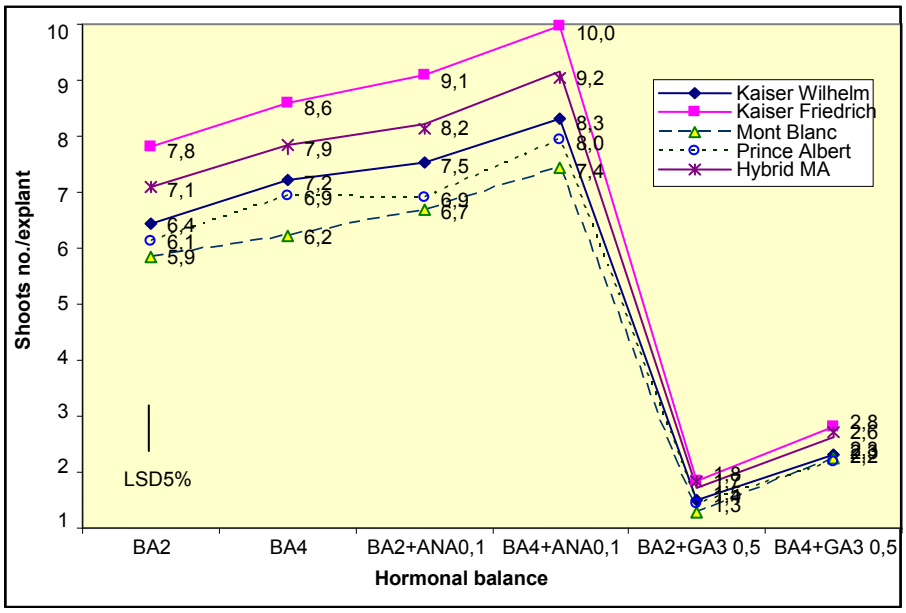

Fig. 1. Shoots number/explant of gloxinia genotypes for different hormonal balance

Table 4

The effect of culture duration on shoots number/explant in gloxinia

Culture duration	Shoots number		Relative values (%)	Difference/ Significance
4 weeks – 2 weeks	5.73	5.49	104.37	0.24
6 weeks – 2 weeks	5.91	5.49	107.65	0.42**
6 weeks – 4 weeks	5.91	5.73	103.14	0.18

$LSD_{5\%}$=0.28 $LSD_{1\%}$=0.36 $LSD_{0.1\%}$=0.46

For all varieties the modification of benzylaminopurine concentration did not significantly influence the number of shoots. Supplementing with ANA0,1 of medium based on BA2 caused a significant increase of shoots formation at varieties: Kaiser Wilhelm, Kaiser Friedrich and MA hybrid, while adding ANA0,1 at medium with BA4 generated a significant increase of this trait in all varieties. The use of gibberellic acid had a significantly lower efficiency compared to α-naphthaleneacetic acid in combination with different benzylaminopurine concentrations, on shoots formation for all varieties (Fig. 1).

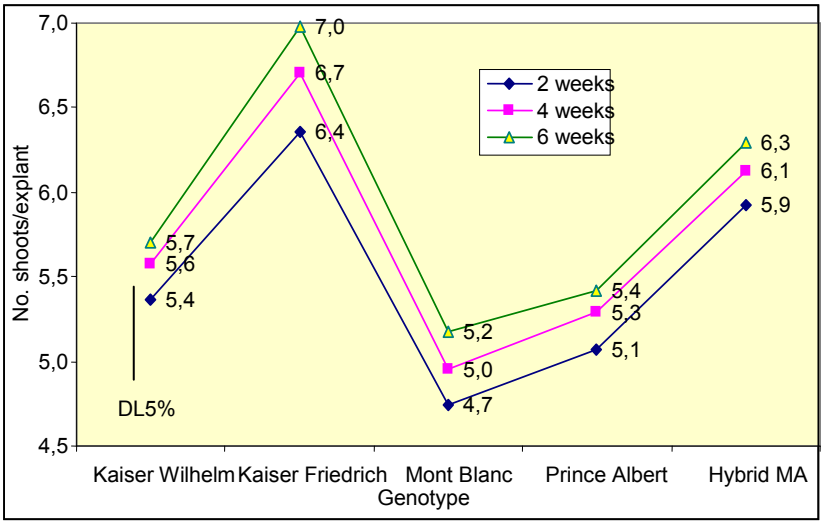

Fig.2. Shoots number/explant of gloxinia genotypes depending on culture duration

Taking into account the dynamics of the number of shoots / explant from the same variety (Fig. 2), it appears that the largest differences were recorded at the variety Kaiser Friedrich, where the extension of culture up to six weeks caused a significant increase of approximately 10% of shoots number compared with the values recorded at two weeks. For the other varieties the culture duration did not significantly influence the formation of shoots.

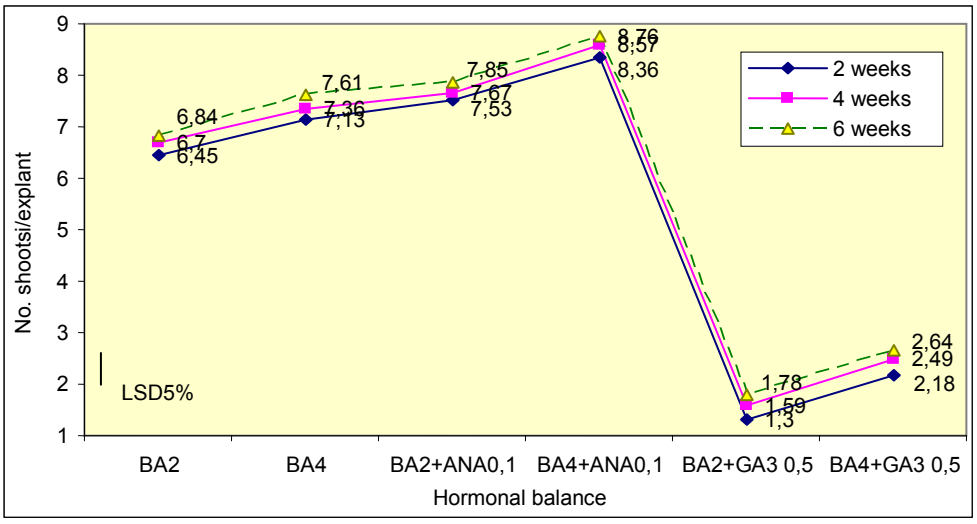

Fig.3. Shoots number/explant of gloxinia genotypes depending on hormone balance and culture duration

Taking into account the cumulative effect of hormonal balance and culture duration on the shoots formation of Gloxinia studied genotypes an amplitude of 6-7 was observed. Thus, the use of the combination of benzylaminopurine 4 mg/l and alpha-naphthylacetic acid 0,1 mg/l has generated a significantly higher number of shoots than the other hormonal balance. Changing the concentration of benzylaminopurine from 2 to 4 mg/l caused a significant increase of this trait, both on simple balance and in mixtures with alpha-naphthylacetic and gibberellic acid. In the hormonal balances based on benzylaminopurine the alpha-naphthylacetic acid had a higher significant effect on the formation of shoots compared with gibberellic acid.

Conclusions

1. The hormonal balance has the highest contribution on the variability of this trait (85.73%), followed by the genotype (5,51 %), while the culture duration had a lower contribution of 2.24 %. Also the combined effects of the factors have significantly influenced the shoots formation;

2. Kaiser Friedrich variety recorded average values of shoots number/explant significantly higher to other genotypes with increases ranging from 9.50% compared to MA hybrid and 25.75% to Mont Blanc variety.

3. The balance BA4+ANA0,1 proved to be the most effective for the initiation and formation of shoots, thus resulting statistically assured increases of shoots number/explant compared to the other studied hormonal combinations, with relative values ranging from 11% compared to BA2+ANA0,1 and 252 % than BA2+GA3 0,5;

4. During the period between the second and sixth week the shoots formation increased significantly with 7.6%. The extension of in vitro culture from two to four weeks has had a statistically uninsured effect leading to an increase of shoots number/explant with 4.4%, or 3.1% in the case of extended from four to six weeks.

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