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Changes in germination parameters of seven sweet basil (*Ocimum basilicum* L.) varieties due to treating with gibberellic and ascorbic acids

Béla BIRÓ-JANKA,[1, 2] Imre-István NYÁRÁDI,[1] Marcel Matei DUDA,[2] Beáta DEMETER,[1] Andrea NAGYGYÖRGY[1]

[1] Sapientia Hungarian University of Transylvania, Cluj-Napoca, Romania,
Faculty of Technical and Human Sciences, Târgu-Mureș, Department of Horticulture
e-mail: bela.biro@ms.sapientia.ro

[2] University of Agriculture and Veterinary Medicine, Faculty of Agriculture,
3–5 Mănăștur Street, Cluj-Napoca, Romania

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Abstract. During the study, the seeds of seven sweet basil cultivars were treated with 100 ppm solutions of gibberellic acid or ascorbic acid prior to a 14-day germination experiment. Values of the first day of germination, germination energy, germinability, mean time and rate of germination, and uncertainty and synchrony of germination were calculated. The results show that both of the substances altered the measured parameters, priming with gibberellic acid proving to be a more effective way to enhance the germination of basil seeds. Also, they revealed major differences among the germination parameters of the examined cultivars.

Keywords: enhance, germinability, priming

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is an aromatic annual herb belonging to the *Lamiacieae* family [1], subfamily *Nepetoideae*, genus *Ocimum* [2]. It is native to Asia (India, Pakistan, Iran, and Thailand) [3]. It is cultivated in the tropics and also in temperate climate in Europe and the Americas [4].

Some species, such as *Ocimum americanum* L, have insecticidal properties, while others have ornamental qualities with a particular leaf shape, size, and colour: e.g. the Purple Ruffles variety of *O. basilicum* [5, 6]. *Ocimum gratissimum* and *Ocimum sanctum* are also cultivated worldwide [7]. Apart from these uses,

basil has been utilized in traditional medicine for curing headaches, diarrhoea, and constipation [8, 9].

The common sweet basil is of high economic importance because of the essential volatile oil derived from its leaves [10–12]. The composition of the essential oil determines the specific aroma of the plant [13], making it a widely used substance in the perfume industry and against several diseases [7, 14]. The essential oil composition varies among different cultivars [15].

Basil has antioxidant [9, 16], antifungal [17], anticancer [18, 19], and antiviral activities [20].

The pharmaceutical, aromatic, and culinary properties of sweet basil are due to its content of different valuable active substances [21, 22]. Basil is a source of phenolic acids such as rosmarinic, caffeic, or cichoric acid and derivatives of lithospermic acid and lithospermic acid B, which help in healing certain renal diseases [16, 23]. Anthocyanins are also highly valued substances with antioxidant activity [24–26].

Low temperature values occurring at the time of germination negatively affect both the germination process and seedling growth [27]. The optimal germination temperature of sweet basil varies between 20 and 30 °C [28–30]. Different treatments and substances can compensate the negative effects of abiotic stress [31]. The effects of gibberellic acid (GA3) on germination parameters of sweet basil were studied. Priming the seeds with GA3 solution prior to germination can reduce the negative effects of drought [32] and salinity [33]. It was reported to have positive effect on the low-temperature germination of different plant species [34]. Ascorbic acid (AsA) is a well-known antioxidant that affects plant growth in many ways such as germination, seedling growth in suboptimal situations [35], or in the case of salinity stress [36].

Therefore, two main objectives were defined: 1. to compare the effect of GA3 and AsA on seven different sweet basil varieties (‘Aromat de Buzău’, ‘Serafim’, ‘Busuioc Dulce’, ‘Italiano Classico’, ‘Dark Opal’, ‘Genovese’, and ‘Grand Verte’) germinating on different temperature values and 2. to compare the germination of the mentioned basil cultivars.

2. Materials and methods

Plant material

The seeds of the seven sweet basil cultivars were obtained from the Buzău Research Station for Legumiculture (SCDL Buzău) in the case of ‘Aromat de Buzău’, ‘Serafim’, ‘Busuioc Dulce’, and ‘Grand Verte’ varieties; the seeds of ‘Italiano Classico’ and ‘Dark Opal’ varieties from S.C. Morami S.R.L. and in the case of the ‘Genovese’ variety from S.C. Agrosem Impex S.R.L.

Priming materials/substances

The gibberellic acid (GA3) was purchased from Duchefa Biochimie.
The ascorbic acid (ASA) was purchased from VWR, part of Avantor.

Experimental design

The experiment was carried out at 15 C° using four replications of each 100 seeds from the seven basil varieties. The seeds were counted out and immersed in the priming solutions (control – containing distilled water, 100 ppm GA3 solution, 100 ppm ASA solution) for 24 hours. After the 24 hour-period, the seeds were placed in Linhardt-dishes using blotting paper as substrate. The lower compartments of the dishes were filled with distilled water and covered with a glass lid to ensure a high and constant relative humidity throughout the germination process.

The dishes were then placed in a growth chamber (Fitotron Poleko, Model: HKK240) for 14 days at a constant temperature and humidity level, without illumination. The batches were verified during data collection, and additional distilled water was administered if needed.

Data collection

The following parameters were recorded during the experiment:

- Time elapsed until the emergence of the first seedling, also called Tindicator of the velocity of the initiation of germination (during the first part of the process) [37].
- Germination energy of the seeds (GE), expressed in %, measured at the halftime of the study (on the 7^{th} day), the same way as in the case of germinability.
- Germinability (G), expressed in %, measured at the end of the study (on the 14^{th} day); it indicates the capacity of the batches to germinate under proper circumstances [38].
- Mean germination time, used to evaluate seedling emergence [39], [38]. The lower the mean germination time, the faster is the germination of a population of seeds completed [37].
- Mean germination rate (MR) is calculated as the reciprocal of mean germination time (MT) [38].
- Uncertainty (U) of the germination process: low values indicate a high synchrony of the germination and vice versa [39].
- Synchrony (Z) value ranges from 0 to 1, 0 meaning that at least two seeds germinated at separate times, while 1 means that all seeds germinated at the same time [38, 39].

The seed batches were verified on a daily basis, and the emerged plantlings were counted. Germination was considered successful if the radicle of the plant has penetrated the seed husk.

Statistical analysis

The collected data were subjected to one-way ANOVA (analysis of variance) carried out with the Paleontological Statistics (PAST) statistical software, version 3.25. After the normality of the data sets had been verified, Tukey pairwise test was carried out in the case of normally distributed data and Mann-Whitney pairwise test in the case of non-normally distributed data (marked with an * in the tables). P values are in the majority of the cases less than 0.001; otherwise, the values are displayed in the tables.

3. Results and discussions

The first day of germination (FDG), or, more precisely, the emergence of the first seedling was positively affected by treating the seeds with GA3, which reduced the time needed until FDG with 1–2 days. AsA treatment also reduced the time of FDG at a smaller scale in the case of 'Aromat de Buzau', 'Busuioc Dulce', and 'Dark Opal'; its values exceeded the control groups' values in the rest of the cases. Due to the lack of variation among the repetition values, no statistical analysis was conducted. For results, see *Table 1*.

Due to invariances among the data collected from 'Aromat de Buzau' in the case of germination energy (GE), statistical analysis could not be run. Priming the seeds with GA3 had positive effect on the GE of the lots. In the case of 'Dark Opal' seeds, none of the priming treatments had significantly different results than the others. Seed lots of 'Serafim', 'Busuioc Dulce', and 'Italiano Classico' varieties responded positively to GA3 priming, the differences being significant. Likewise, control and AsA treatments did not differ from each other. The seeds of the 'Grand Verte' variety responded positively to both GA3 and AsA treatments, having the same results regarding GE, while the control group produced significantly lower results. The 'Genovese' variety was the only case where the AsA treatment had significantly lower results than the control group (*Table 1*).

'Serafim' and 'Dark Opal' varieties showed no significant differences among the treatments regarding germinability. In the cases of 'Aromat de Buzau' and 'Busuioc Dulce' varieties, the GA3 treatment had a significantly positive effect in comparison to the control treatment. 'Italiano Classico' and 'Genovese' responded negatively to AsA treatment, the differences being significant; these varieties also showed no differences between control and GA3 treatments. Only

the seeds of the 'Grand Verte' variety germinated at a significantly higher rate under AsA treatment. Results are shown in *Table 1*.

Studying the results of the mean time of germination reveals a relative similarity in the way how the different seed lots reacted to the treatments. In all of the cases, except the 'Italiano Classico' and 'Grand Verte' varieties, the results indicate that the AsA treatment was not significantly different from the control group, lots treated with GA3 germinating in a significantly less time. In the case of 'Italiano Classico' and 'Grand Verte' seedlots, it can be said that both treatments resulted in the reduction of the time needed for germination (*Table 1*).

A relative similarity can be observed when comparing the results of the rate of germination, too. The obtained data of the rate of germination are similar to the results of the mean time of germination. The seeds of all varieties responded positively to the GA3 treatment, the rate of germination values being significantly higher than in the case of the control group and, except the 'Italiano Classico' and 'Grand Verte' varieties, upon AsA treatment. Results are shown in *Table 1*.

Comparing the results of uncertainty, differences occur between the ways how the varieties responded to the different treatments. There are no significant differences between the treatments in the case of the 'Aromat de Buzau', 'Serafim', and 'Dark Opal' varieties. Priming with GA3 or AsA, the 'Busuioc Dulce', 'Genovese', and 'Grand Verte' seeds resulted in a significant reduction of uncertainty values compared to the control group. The analysis of the uncertainty values of 'Italiano Classico' seed lots reveals that priming with AsA has reduced them significantly compared to the control group; results from seeds treated with GA3 do not differ from the other two treatments (*Table 1*).

The treatments had different effects on the synchrony of germination, the seeds reacting to them in alternative ways. In the case of 'Busuioc Dulce', 'Genovese', and 'Grand Verte' seeds, priming with AsA resulted in higher synchrony values, the differences being significant in comparison to the control group; at the same time, 'Genovese' seeds treated with GA3 also produced significantly higher results than the control group, while in the case of 'Busuioc Dulce' and 'Grand Verte' the synchrony of seeds primed with GA3 did not differ from the other two treatments. The synchrony of 'Serafim', 'Italiano Classico', and 'Dark Opal' seeds showed no significant differences. In the case of the 'Italiano Classico' variety, the synchrony of germinating seeds was the highest in the lot belonging to the control group (*Table 1*).

Table 1. The results of the one-way ANOVA analysis and the Tukey post-hoc test, the treatments being compared (in the case of the * Mann-Whitney test)

'Aromat de Buzau'	FDG (day)	GE (%)	G (%)	MT (day)	MR (day^{-1})	U (bit) *	Z p< 0.05
Control	8	0	24.25^{b}	10.751^{a}	0.093^{b}	2.428^{a}	0.181^{ab}
GA3	6	8	46^{a}	9.133^{b}	0.109^{a}	2.556^{a}	0.212^{a}
AsA	7.75	0.25	17^{b}	10.731^{a}	0.093^{b}	2.527^{a}	0.128^{b}
'Serafim'	**FDG (day)**	**GE (%) p<0.01**	**G (%)**	**MT (day)**	**MR (day^{-1})**	**U (bit)**	**Z**
Control	4.5	51^{b}	83.4^{a}	7.448^{a}	0.134^{b}	2.539^{a}	0.208^{a}
GA3	3.75	74.5^{a}	89.75^{a}	5.838^{b}	0.171^{a}	2.551^{a}	0.199^{a}
AsA	5	38^{b}	82.5^{a}	8.320^{a}	0.120^{b}	2.616^{a}	0.188^{a}
'Busuioc Dulce'	**FDG (day)**	**GE (%) p<0.01**	**G (%) p<0.05**	**MT (day)**	**MR (day^{-1})**	**U (bit) p<0.01**	**Z p<0.01**
Control	5.5	39.5^{b}	84.25^{b}	8.163^{a}	0.123^{b}	2.720^{a}	0.171^{b}
GA3	3	90.75^{a}	98.25^{a}	5.682^{b}	0.176^{a}	2.299^{b}	0.231^{ab}
AsA	5.25	61.75^{b}	88^{ab}	7.376^{a}	0.135^{b}	2.216^{b}	0.272^{a}
'Italiano Classico'	**FDG (day)**	**GE (%) * p < 0.05**	**G (%)**	**MT (day) p < 0.05**	**MR (day^{-1}) p<0.05**	**U (bit)**	**Z p<0.05**
Control	4.5	25^{b}	52.75^{a}	8.2450^{a}	0.122^{b}	2.777^{a}	0.164^{a}
GA3	3	53.75^{a}	63.75^{a}	5.895^{c}	0.170^{a}	2.453^{ab}	0.218^{a}
AsA	5	19.5^{b}	25.75^{b}	6.949^{b}	0.144^{ab}	2.308^{b}	0.222^{a}
'Dark Opal'	**FDG (day)**	**GE (%)**	**G (%)**	**MT (day)**	**MR (day^{-1})**	**U (bit)**	**Z**
Control	4.5	44^{a}	67.75^{a}	7.190^{a}	0.139^{b}	2.468^{a}	0.211^{a}
GA3	3.5	51.5^{a}	67.75^{a}	5.742^{b}	0.174^{a}	2.185^{a}	0.257^{a}
AsA	4	50.5^{a}	66.75^{a}	6.931^{a}	0.144^{b}	2.600^{a}	0.197^{a}
'Genovese'	**FDG (day)**	**GE (%)**	**G (%) * p<0.05**	**MT (day) * p<0.05**	**MR (day^{-1}) * p<0.05**	**U (bit) p<0.01**	**Z p<0.01**
Control	3.25	76^{b}	92^{a}	6.083^{a}	0.164^{b}	2.508^{a}	0.197^{b}
GA3	3	91.25^{a}	94.5^{a}	4.763^{b}	0.210^{a}	1.976^{b}	0.295^{a}
AsA	5	52^{c}	61^{b}	6.201^{a}	0.161^{b}	2.0434^{b}	0.306^{a}
'Grand Verte'	**FDG (day)**	**GE (%) ***	**G (%) p<0.05**	**MT (day)**	**MR (day^{-1}) p<0.01**	**U (bit)**	**Z**
Control	3.5	40.75^{b}	72^{b}	7.414^{a}	0.135^{b}	2.518^{a}	0.200^{b}
GA3	3.25	71.5^{a}	77.25^{ab}	5.292^{b}	0.191^{a}	1.883^{b}	0.317^{ab}
AsA	4	71.5^{a}	82.75^{a}	5.815^{b}	0.172^{ab}	1.841^{b}	0.343^{a}

FDG – first day of germination, GE – germination energy, G – germinability, MT – mean time of germination, MR – mean rate of germination, U – uncertainty, and Z – synchrony of germination. Different letters denote significant differences; in the case of the lack of statistical analysis, no letters appear.

Comparing the values of germination parameters of the sweet basil varieties

The seeds of the ‘Aromat de Buzau’ cultivar stand out by needing almost twice as much time until FDG as the rest of the cultivars regardless the treatment applied to them. Among the untreated lots, ‘Genovese’ and ‘Grand Verte’ seeds had the lowest FDG values: they needed only 3.25 and 3.5 days until the first seedlings appeared. After priming them with GA3, none of the sweet basil varieties’ seeds had differences among them; AsA treatment had a slight effect on ‘Aromat de Buzau’, ‘Dark Opal’, and ‘Genovese’ seeds, and it had no positive effect on the rest of the cultivars (*Table 2*).

Among the untreated seed lots, the ‘Genovese’ variety had the highest GE, followed by ‘Serafim’ and ‘Dark Opal’. Among the lots treated with GA3, the top three cultivars were ‘Genovese’, ‘Busuioc Dulce’, and ‘Serafim’; in the case of AsA treatment, the ‘Grand Verte’, ‘Busuioc Dulce’, and ‘Genovese’ cultivars had the highest values. The germination energy (GE) of the cultivars changed in a positive way in the case of both priming materials, the number of seed lots with GE values exceeding 50%, rising to 4 in the case of the AsA treatment and to 6 in the case of GA3 treatment, from an initial 1 in the case of the control group. ‘Busuioc Dulce’ and ‘Genovese’ seed lots had GE values over 90% after being treated with GA3; on the other hand, the seeds of ‘Italiano Classico’ variety’s GE values were lower than the control groups. Again, ‘Aromat de Buzau’ differs from the other cultivars by having GE values under 10% (*Table 2*).

The varieties from the untreated group with the highest G values are the following: ‘Genovese’, ‘Busuioc Dulce’, and ‘Serafim’; from the GA3-treated group: ‘Busuioc Dulce’, ‘Genovese’, and ‘Serafim’; from the AsA-treated group: ‘Busuioc Dulce’, ‘Serafim’, and ‘Grand Verte’. The priming of seeds with GA3 resulted in the growth of germinability values in all of the varieties except ‘Dark Opal’, where absolutely no change occurred. On the other hand, treating the seeds with AsA had a different effect on them, ‘Aromat de Buzau’, ‘Italiano Classico’, ‘Dark Opal’, and ‘Genovese’ responding to the treatment with lower G values. The varieties having the lowest germinability in all cases were ‘Aromat de Buzau’ followed by ‘Italiano Classico’ (*Table 2*).

Table 2. The results of the one-way ANOVA analysis and the Tukey post-hoc test, the varieties being compared (in the case of the * Mann-Whitney test)

Control	FDG (day)	GE (%)	G (%)* p<0.05	MT (day)* p<0.05	MR (day^{-1}) p<0.01	U (bit)	Z
Aromat de Buzău	8	0	24.25 ^{e}	10.752 ^{a}	0.093 ^{c}	2.428 ^{a}	0.181 ^{a}
Serafim	4.5	47.75	80.5 ^{abc}	7.571 ^{bc}	0.132 ^{b}	2.612 ^{a}	0.197 ^{a}
Busuioc Dulce	5.5	39.5	84.25 ^{ab}	8.163 ^{b}	0.123 ^{b}	2.720 ^{a}	0.171 ^{a}
Italiano Classico	4.5	25	52.75 ^{d}	8.245 ^{b}	0.122 ^{b}	2.777 ^{a}	0.164 ^{a}
Dark Opal	4.5	44	67.75 ^{c}	7.190 ^{c}	0.139 ^{b}	2.468 ^{a}	0.211 ^{a}
Genovese	3.25	76	92.00 ^{a}	6.083 ^{d}	0.164 ^{a}	2.508 ^{a}	0.197 ^{a}
Grand Verte	3.5	40.75	72 ^{bc}	7.414 ^{bc}	0.135 ^{b}	2.518 ^{a}	0.200 ^{a}
GA_3	FDG (day)	GE (%)* p<0.05	G (%)* p<0.05	MT (day)* p<0.05	MR (day^{-1})* p<0.05	U (bit) p<0.01	Z p<0.05
Aromat de Buzău	6	8 ^{d}	46 ^{d}	9.133 ^{a}	0.109 ^{c}	2.556 ^{a}	0.212 ^{bc}
Serafim	3.75	74.5 ^{b}	89.75 ^{b}	5.838 ^{b}	0.171 ^{b}	2.551 ^{a}	0.199 ^{bc}
Busuioc Dulce	3	90.75 ^{a}	98.25 ^{a}	5.682 ^{b}	0.176 ^{b}	2.299 ^{ab}	0.231 ^{abc}
Italiano Classico	3	53.75 ^{c}	63.75 ^{c}	5.895 ^{b}	0.170 ^{b}	2.453 ^{a}	0.218 ^{bc}
Dark Opal	3.5	51.5 ^{c}	67.75 ^{c}	5.742 ^{b}	0.174 ^{b}	2.185 ^{ab}	0.257 ^{abc}
Genovese	3	91.25 ^{a}	94.5 ^{ab}	4.763 ^{c}	0.210 ^{a}	1.976 ^{b}	0.295 ^{ab}
Grand Verte	3.25	71.5 ^{b}	77.25 ^{c}	5.292 ^{bc}	0.191 ^{ab}	1.883 ^{b}	0.317 ^{a}
AsA	FDG (day)	GE (%) p<0.05	G (%)* p<0.05	MT (day)* p<0.05	MR (day^{-1}) p<0.01	U (bit) p<0.05	Z p<0.05
Aromat de Buzău	7.75	0.25 ^{e}	17 ^{c}	10.731 ^{a}	0.093 ^{e}	2.527 ^{a}	0.128 ^{d}
Serafim	5	48.2 ^{c}	84.6 ^{a}	7.384 ^{b}	0.151 ^{d}	2.578 ^{a}	0.196 ^{cd}
Busuioc Dulce	5.25	61.75 ^{b}	88 ^{a}	7.376 ^{c}	0.135 ^{cd}	2.216 ^{ab}	0.272 ^{abc}
Italiano Classico	5	19.5 ^{d}	25.7 ^{c}5	6.949 ^{d}	0.144 ^{c}	2.308 ^{ab}	0.222 ^{bc}
Dark Opal	4	50.5 ^{b}	66.75 ^{b}	6.931 ^{d}	0.144 ^{bc}	2.600 ^{a}	0.197 ^{cd}
Genovese	5	52 ^{b}	61 ^{b}	6.201 ^{de}	0.161 ^{ab}	2.043 ^{b}	0.306 ^{ab}
Grand Verte	4	77.5 ^{a}	82.75 ^{a}	5.815 ^{e}	0.172 ^{a}	1.841 ^{b}	0.343 ^{a}

FDG – first day of germination, GE – germination energy, G – germinability, MT – mean time of germination, MR – mean rate of germination, U – uncertainty, and Z – synchrony of germination. Different letters denote significant differences; in the case of the lack of statistical analysis, no letters appear.

The varieties from the untreated group with the highest G values are the following: ‘Genovese’, ‘Busuioc Dulce’, and ‘Serafim’; from the GA3-treated group: ‘Busuioc Dulce’, ‘Genovese’, and ‘Serafim’; from the AsA-treated group: ‘Busuioc Dulce’, ‘Serafim’, and ‘Grand Verte’. The priming of seeds with GA3

resulted in the growth of germinability values in all of the varieties except ‘Dark Opal’, where absolutely no change occurred. On the other hand, treating the seeds with AsA had a different effect on them, ‘Aromat de Buzau’, ‘Italiano Classico’, ‘Dark Opal’, and ‘Genovese’ responding to the treatment with lower G values. The varieties having the lowest germinability in all cases were ‘Aromat de Buzau’ followed by ‘Italiano Classico’ (*Table 2*).

Comparing the mean time for germination (MT) of the different groups, it can be said that the ‘Aromat de Buzau’ variety’s seeds needed the longest time to germinate in all cases, the differences being significant. On the contrary, ‘Genovese’ MT values were the lowest (its seeds germinated at a faster rate) among the untreated and GA3-treated groups, while in the AsA-treated group ‘Grand Verte’ had the lowest values. Results show that both GA3 and AsA decreased the MT values of all of the varieties, GA3 treatment exerting a more powerful effect (*Table 2*).

Similar to the MT are the mean rate (MR) values of the seed lots, ‘Aromat de Buzau’ having the lowest and ‘Genovese’ as well as ‘Grand Verte’ the highest MR values. GA3 treatment enhanced the mean rate of germination among all varieties, priming with AsA being less effective, in the case of ‘Genovese’ seeds producing even lower values than the untreated group (*Table 2*).

The comparison of the uncertainty of germination (U) of the cultivars showed no significant differences in the untreated group, ‘Aromat de Buzau’ seeds having the lowest and ‘Italiano Classico’ seeds the highest values. In the group treated with GA3, ‘Genovese’ and ‘Grand Verte’ seeds had significantly lower values than ‘Aromat de Buzau’, ‘Serafim’, and ‘Italiano Classico’ seeds; ‘Busuioc Dulce’ and ‘Dark Opal’ did not differ significantly from the other varieties. After the AsA treatment, seeds with lowest U values were ‘Genovese’ and ‘Grand Verte’, differing significantly from the seeds with the highest U values, which were ‘Dark Opal’ and ‘Serafim’ (*Table 2*).

Comparing the synchrony (Z) of the untreated seeds shows no significant differences, ‘Italiano Classico’ having the lowest and ‘Dark Opal’ the highest values. Priming the seeds with GA3 resulted in a growth of Z values of all varieties, ‘Aromat de Buzau’, ‘Serafim’, and ‘Italiano Classico’ seeds having a lesser and ‘Grand Verte’ a significantly higher value. After treating the seeds with AsA, the ‘Aromat de Buzau’, ‘Serafim’, and ‘Dark Opal’ varieties had the lowest and ‘Genovese’ and ‘Grand Verte’ the highest Z values (*Table 2*).

4. Conclusions

After the analysis of the obtained data, it can be said that GA3 applied in a 100 ppm concentration at 15 °C has a positive effect on the germination parameters of sweet basil. On the contrary, seeds of the different cultivars treated

with AsA responded in adverse ways, AsA enhancing the germination of some of the cultivars and reducing it in other cases.

Comparing the sweet basil varieties shows differences among them, too, 'Aromat de Buzau' seeds having inferior results in most aspects. The 'Serafim', 'Busuioc Dulce', and 'Genovese' varieties had the highest values on average.

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