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THE ASSESSMENT OF SEEDLING QUALITY AIMED TO ENSURE SUSTAINABLE CABBAGE SEED PRODUCTION

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Abstract

Brassicaceae vegetables have been grown and consumed worldwide since ancient times and they are considered a functional food due to the presence of phytochemicals, mineral and vitamins necessary for human well-being. For this reason, species as cabbage are highly requested by consumers. At farm level, constrains related to seed production process highlight the importance of seed germination and seedling performance. The study is integrated in major research aimed to develop new modern scheme for cabbage seed production. The objective of this work was to assess seedling quality by measuring several morphological, phenological and physiological parameters with influence in performance of cabbage sustainable seedling production. The experiment was assessed on "Silviana" autumn cabbage, created at VRDS Bacau, and was conducted using a randomized complete block design with three replications. The work presents the results of germination, seedling growth dynamic, carotenes, xanthophylls and chlorophylls content as affected by different factors as variety, age and growing climate. This study reveals the importance of using qualitative seedlings during the process of seed production.

***Key words**: Brassica, carotenes, xanthophylls, chlorophylls, growth.*

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is one of the most essential agricultural crops in the *Brassicaceae* family, which covers many important vegetable crops such as cabbage, kale, Brussels sprouts, cauliflower, broccoli, and kohlrabi and therefore has an ancient legacy of agriculture practices.

Cabbage is a leafy white biennial used as an annual vegetable because of its densely leafed (compact) heads. Cultivars come in a variety of forms, colours, and leaf textures.

Cabbage plants are classified as crinkled-leaf, loose-head or smooth-leaf, firm-head plants based on their leaf type.

Cultivated cabbage cultivars have a wide range of morphological features and are divided into two main groups: white (*Brassica oleracea* var. *capitata* f. *alba*), which is the common name for cabbage with green leaves, and red (*Brassica oleracea* var. *capitata* f. *rubra*), which is the common name for cabbage with red leaves (Singh et al., 2006).

Cabbage head is a large bud that contains the majority of the plant's leaves. It has rather low temperature needs; the biological threshold cannot be decreased any further than 5 and 6°C, and the ideal temperature for vegetation is set between 15 to 18°C. It can even withstand negative temperatures for a limited duration (Iosob et al., 2019).

Cabbage is frequently consumed because of its bioactive and antioxidant activities, along with anti-inflammatory and antibacterial characteristics (Cartea et al., 2011; Šamec et al., 2011).

Natural pigments give fruits and vegetables their distinctive colour, hence chlorophylls predominate in white cabbage cultivars (Fernández-León et al., 2010), and anthocyanins dominate in red cabbage cultivars, the colour of fruits and vegetables is primarily determined by their presence (Arapitsas et al., 2008).

Thus, according Lisiewska et al. (2004), the level and ratio of chlorophyll pigments have a significant impact on colour attraction. Colour is perhaps the most important attribute engaged in consumers' evaluations of products, according to Tijskens et al. (2001), and it plays a major contribution in the product's acceptability.

Chlorophylls are often accompanied along with carotenoid pigments, which range spectrum colour from yellow to orange (Kidmose et al. 2001).

Chlorophylls are pigments that give plants and fruits their green colour and play an important part in photosynthesis. Vegetables' chlorophyll content is important for both their favourable development and their aesthetic attractiveness (Bulgari, 2017).

Its biodegradation is linked to cellular break-down and/or senescence, and it's frequently used to determine green vegetable quality loss (Hodges et al., 2000; Toivonen and Sweeney, 1998).

In fact, there has been evidence of a substantial link between chlorophyll concentration and overall aesthetic quality of vegetables (Aguero et al., 2008).

Furthermore, chlorophyll can be classified as a bioactive chemical since its naturally occurring dietary derivatives that have antioxidant and antimutagenic properties (Hsu et al., 2008; Ferruzzi et al., 2002; Ferruzzi and Blakeslee, 2007).

Carotenoids are important nutritional and functional elements. Green vegetables provide a reliable source of carotenoids, with cole crops being one of the most prevalent (Kalt 2005). β-carotene is among the most useful of the carotenes, evidencing pro-vitamin A activity (Nishino et al., 1999).

α-carotene, β-carotene, capsanthin, lutein, lycopene, and zeaxanthin are common carotenoids in plants. Carotene is a vitamin A precursor, and carotenoids with β rings have the highest provitamin A activity. In mammals, provitamin A carotenoids are converted to retinol and other linked retinols, which are involved in the visual cycle and gene control. (Rodríguez-Concepción, 2018).

Antioxidant compounds such as chlorophyll and carotenoids are found in cole crops (Kidmose et al. 2001).

With the exception of polyphenols, the major bioactive ingredient in *Brassicaceae* plants is vitamin C, which is among the most important micronutrients (Martín-Belloso and Fortuny, 2011). Pigment molecules as chlorophylls, carotenoids, and anthocyanins are also expressed when there is significant biological activity (Hsu et al., 2013).

Antioxidants are compounds that can cease or slow the lipid oxidation or other molecules by inhibiting or postponing the beginning or multiplication of oxidative chain reactions (Emmons et al., 1999; Velioglu et al., 1998).

Crop production, yield and quality are a critical concern in vegetable cultivation that is garnering more attention (Jablonska-Coglarek and Rosa 2002).

One of the most significant inputs in high-yield vegetable crop development and in seed production is the seedling quality. High-quality seedlings are required for proper stand establishment and homogenous plant growth. Traditionally, nursery production has centred on growing seedlings in a cost-effective and efficient manner. Presently, there is an increasing concern in decreasing seedling production's environmental impact.

Seeds encounter a variety of issues in terms of production, postharvest storage, and future quality. Furthermore, given the effects of global warming as a symptom of climate change, various stress factors may result in poor seed effectiveness in terms of decreased germination, imbalanced seedling emergence, poor seedling establishment, and counterpro-ductive modifications of root cell architecture, resulting in a significant yield reduction (Rakshit and Singh, 2018).

Common germination evaluations can be used to determine seed performance in the field (Johnson and Wax, 1978), but subsequent establishment and growth projections are not always factual (Heydecker, 1972).

The success of producing healthy and well-growing seedlings is heavily influenced by the quality and germinability of seeds. Different production strategies can improve germinability and seedling health (Himanen and Nygren, 2015).

Seed quality, germination and early seedling vigour are critical factors in the success among many crops. Environmental variables such as temperature and humidity influence the germination of most seeds (Chachalis and Reddy, 2000; Taylorson, 1987).

The ability for germination and the rate of seed germination are affected by temperature, whereas soil moisture is required for germination (Bewley and Black, 1994). Germination is hampered by a lack of moisture. Early seedling survival is influenced by the moisture level of the growth medium. The influence of relative humidity on seedling development in most plants is similarly as significant (Went, 1958). The aim of this study was to evaluate seedling quality by assessing a variety of morphological, phenological, and physiological characteristics that have an impact on the performance of cabbage seedling production.

MATERIALS AND METHODS

Plant material

In the experiment, the autumn white cabbage variety "Silviana" patented by the Vegetable Research and Development Station Bacau, was utilised in the trials required to fulfil the specified aim.

"Silviana" cultivar is an autumn cabbage type that is highly resistant to cracking and has a high yield potential. The leaves have a fine texture, the head form in longitudinal section is round to elliptical, and the colour of the leaves is raw green to medium intensity green.

It also contains a vitamin C concentration of 44.50 mg per 100 g, a cellulose level up to 1.15% and a production potential of 100 to 120 t ha^{-1}. The height of roughly 18-20 cm, the circumference of 18-21 cm, the average weight of 1.6-2.7 kg, the lack or very faint presence of anthocyanin colour of the covering leaves and the yellow tint of the inside leaves are further characteristics of the vegetative bud.

Site description

The studies were arranged and carried out in the VRDS experimental greenhouse which is situated in the north-eastern section of Bacau, on a river terrace with an altitude of 5-7 meters and a height of 165 meters above sea level, in the Bistrița - Siret interfluve, about 4 kilometres north of their confluence.

Experimental design

Three distinct dates were chosen for sowing. The first took place on 27 May 2021, followed by the second on June 28, 2021, and the third on 30 July 2021, in plastic pots filled with commercial peat-based potting material. The pots were arranged in a greenhouse compartment on rolling benches.

The experiment was carried out using a three-replication randomized complete block design. The plants were equally watered as needed with tap water (usually one time per day) by overhead misting after seedling emergence. A 40-day-old cabbage seedling was used for the trials, which was sowed in alveolar palettes according to the three epochs stated above.

After 40 days, when the plants had achieved proper planting age, the cabbage samples were removed from the greenhouse and transported to the laboratory for analysis. Plant samples were hand-picked at random followed by assessment of several morphological traits.

Germination

For a control test ISTA regulations were followed when conducting germination testing (International Seed Testing Association).

On Petri dishes with filter paper wet with tap water, three replicas of 100 seeds each lot were sowed. The seeds were germinated on a germinator for 16 hours in darkness and 8 hours in white fluorescent light, respectively, at alternating temperatures of 20 °C and 30 °C. The final germination percentage (FGP) was calculated. For each period the germination capacity was assessed in greenhouse conditions, by counting the days from sowing to emergence.

Pigments determination

The spectrophotometric method was applied, which is quick and readily available, with the BOECO S-20 Spectrophometer. 1g of fresh sample (leaves fresh tissue) was extracted with 80% acetone (v/v). Successive extractions were performed until the residue has become colourless and then was adjusted to 100 ml fixed volume. The absorbance of extracts was measured at 663, 646 and 470 nm against blank (acetone 80%). Formulas used for calculation of pigments content were those developed by Mackinney (1941) and the values were expressed in mg $100g^{-1}$:

$$\text{Chlorophyll a} = [(12{,}21 \times DO_{663}) - (2{,}81 \times DO_{646})] \times 5$$
$$\text{Chlorophyll b} = [(20{,}13 \times DO_{646}) - (5{,}03 \times DO_{663})] \times 5$$
$$\text{Carotenoids and xanthophylls} = [(1000 \times DO_{470}) - (3{,}27 \times \text{Chl. a}) - (1{,}04 \times \text{Chl. b})] / 229$$

Seedling growth dynamic

The seedlings morphological characters analysis was performed on all 3 sets of plants at the age of 40 days, each set being represented by a sowing period. Determinations have been made in this regard on the thickness of the seedling at the stem base (mm), the height of the seedling at planting maturity (cm), number of leaves, total seedling weight (g), weight of aerial part of the seedling (g), and on roots weight (g), using the ruler, scale (Radwag PS 600/C/2) and the caliper.

Statistical analysis
Statistical methods were used to evaluate the collected morphological data. The evaluation of the obtained analytical data was performed by statistical means. The IBM SPSS Statistics programme, version 26.0, was used for statistical analysis. The ANOVA test was used to examine the differences between the means, followed by the Tukey's posthoc test. At the threshold of $P < 0.05$, a significant difference was evaluated. The results were reported as means ± standard errors.

RESULTS AND DISCUSSIONS

In the recent period a significant information on how to produce high-quality seedlings was developed. The production of well-growing seedling is mandatory, because of its influence in crop competitiveness in ever-changing environment. Our focus was on investigation of seedling morphology and a few physiological parameters as influenced by different sowing periods. The general objective is to develop new modern scheme for cabbage seed production, seedling management being one important link of the process.

Table 1. The results obtained from the analysis of some environmental conditions

Sowing time	Light intensity (W / m^2)	Atmospheric humidity (%)
Period 1 (27.05.2021)	214.16±10.29ab	79.56±1.49a
Period 2 (28.06.2021)	238.21±7.02a	74.48±1.18b
Period 3 (30.07.2021)	206.82±8.61b	74.27±1.34b

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Table 1 shows the differences in terms of light intensity and atmospheric humidity for each sowing period. The first period was sown on the 27.05.2021 and on 31.05.2021 plants had emerged; thus 4 days were needed for emergence. For the second period the sowing took place on 28.06.2021 and 3 days later, on 01.07.2021 the seedlings started to emerge. In last period, the third one, the emergence took place in 3 days, with a sowing date on 30.07.2021 and an emergence date on 02.08.2021.
The outcomes of the analyses done on the morphological characters of white cabbage seedlings according to the sowing period is reported in the following section.

Table 2. The results obtained from the analysis of some morphological characters of seedlings

Sowing time	The diameter of the seedling at the stem base (mm)	The height of the seedling at planting maturity (cm)	Number of leaves
Period 1 (27.05.2021)	3.88±0.11a	18.51±0.75a	5.60±0.26a
Period 2 (28.06.2021)	3.43±0.12b	18.61±0.64a	4.90±0.18ab
Period 3 (30.07.2021)	3.21±0.10b	15.85±0.51b	4.70±0.15b

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Table 3. The results obtained from the analysis of some morphological characters of seedlings

Sowing time	Total seedling weight (g)	Weight of aerial part (g)	Roots weight (g)
Period 1 (27.05.2021)	5.30±0.27a	5.11±0.28a	0.188±0.011a
Period 2 (28.06.2021)	4.80±0.24a	4.62±0.23a	0.186±0.009a
Period 3 (30.07.2021)	3.53±0.17b	3.40±0.17b	0.137±0.009b

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Tables 2 and 3 shows the differences obtained from the analysis of morphological characters performed on seedlings at the age of 40 days, for each sowing period. Our interest was to detect the most suitable sowing period to ensure the development of high robust seedlings. The assessment of robustness included thickness, expressed by measuring the diameter of stem base, seedling height at planting maturity, number of developed leaves in a specific period, total fresh weight of seedlings, roots and aerial part.
Measurements of stem base diameter showed a small variation of the mean value of this parameter between three investigated periods. The average values identified ranged from 3.21 mm (period 3) to 3.88 mm (period 1).
When comparing the height of the seedling at planting maturity from all the three periods, we

can observe that the average varied between 15.85 cm in period 3 (30.07.2021) and 18.61 cm in period 2 (28.06.2021), with the highest consistency reported in period 2. In terms of the number of leaves the average value fluctuated between 4.70 and 5.60, with the highest value recorded in period 1. For the first sowing period, it can be seen a significantly greater development of the plant's aerial components based on these preliminary data.

Regarding the total weight of seedlings, significant variations of the average per period can be observed, with a maximum of 5.30 g recorded in the first period and a minimum of 3.53 g in the third period.

In specific cases, fresh weight of the above ground section of seedlings taken under observation, average values ranged from 3.40 g to 5.11 g, similarly to total weight of seedlings, period 1 recorded the highest value, while period 3 recorded the lowest.

The root mass averages obtained fluctuated between 0.188 g and 0.137 g, with 0.188 g recorded in period 1 and followed closely by period 2 with a mean value of 0.186 g and lastly 0.137 g obtained by period 3.

All traits were investigated during the juvenile period. The seedling phase and the post-planting phase are included in this period which is characterized by intense vegetative growth, that is correlated with the value of temperature, soil moisture and the degree of supply of plants with mineral substances. At suboptimal temperatures and in conditions of inadequate supply of water and minerals, leaf growth is inhibited. During this period the ambient factors cannot determine the floral induction.

Our preliminary results confirm the fact that the first sowing period led to the best growing process of seedlings. In a period of 40 days, the seedlings reached the average of total weight of 5.30 g, the stem base diameter of 3.88 mm, the mean height at planting maturity of 18.51 cm, an average of total number of leaves of 5.6, the weight of aerial part of 5.11 g, and an average of 0.188 g for the weight of the roots.

Chlorophyll content (mg/100 g fresh tissue) of cabbage seedlings at different sowing stages are presented in Table 4.

Chlorophyll ratio, carotenoids and xanthophylls content and ratio (mg/100 g fresh tissue) of cabbage seedling at different sowing are presented in Table 5.

Table 4. Chlorophyll content (mg/100g fresh tissue)

Sowing time	Chlorophyll A	Chlorophyll B	Total chlorophyll A+B
Period 1 (27.05.2021)	7.08±0.79b	8.14±0.87b	15.22±1.29b
Period 2 (28.06.2021)	8.67±0.55ab	7.16±1.14b	15.83±1.01b
Period 3 (30.07.2021)	10.49±1.14a	25.71±1.33a	36.20±2.03a

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Table 5. Carotenoids and Xanthophylls content and chlorophyll ratio (mg/100g fresh tissue)

Sowing time	Carotenoids and Xanthophylls C X	Chlorophyll ratio A/B	Total chlorophyll / Carotenoids and Xanthophylls ratio
Period 1 (27.05.2021)	3.70±0.38c	0.93±0.09b	4.24±0.22b
Period 2 (28.06.2021)	2.05±0.28b	1.63±0.31a	10.73±2.94a
Period 3 (30.07.2021)	5.57±0.28a	0.41±0.04b	6.49±0.17ab

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Chlorophyll has a significant role in photosynthesis, and light regime is essential factor for chlorophyll synthesis.

Tables 4 and 5 show the differences obtained from the analysis of leaf pigments performed on 3 sets of seedlings aged 40 days, each set represented by a sowing period.

The average amount of chlorophyll A pigments ranged between 7.08 mg/100 g and 10.49 mg/ 100 g, with the highest value being recorded by seedlings from period 3 and the lowest value being reported by period 1.

It is observed that in the case of chlorophyll B, the average varied from 7.16 mg/100 g fresh tissue to 25.71 mg/100 g, with the highest value set by period 3 and the lowest value by period 2.

In terms of total chlorophyll pigments, the period with the sowing date of 30.07.2021, namely period 3, had the highest mean with a maximum value of 36.20 mg/100 g.

Therefore, in the case of seedlings from period 3, the average content of carotene and xanthophylls reached the maximum of 5.57 mg/ 100 g fresh tissue.

In the case of the chlorophyll A/chlorophyll B ratio, the greatest average of 1.63 mg/100 g was marked by period 2.

The average ratio of total chlorophyll/carotene and xanthophyll fluctuated between 4.24 mg/ 100 g and 10.73 mg/100 g, with the highest number being reported by period 2 with the date of sowing on 28.06.2021.

The preliminary data obtained from the cabbage seedling study revealed significant variance between sowing periods.

In view of the findings, we may conclude that the third period produced the highest levels of chlorophyll A and B pigments, as well as carotenoids pigments.

CONCLUSIONS

The research on seedling quality will support the development of modern seed production technology and will allow and facilitate the development of criteria for easy evaluation and identification of competitive planting material.

Based on these preliminary data, we observed that the first sowing period ensured significantly greater development of the plant's aerial components: number of leaves, the height of the seedling at planting maturity, the diameter of the seedling at the stem base and weight of the aerial part.

This can be explained by temperature, light and humidity regime in favour of vegetative growing of cabbage seedlings.

The preliminary data of cabbage seedling chlorophyll investigation revealed significant variance between sowing periods, third period produced the highest levels A and B chlorophyll pigments as well as carotenoids pigments.

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