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# Evaluation of virus-free potato minitubers derived from *"in vitro"* meristem cultures for high-quality seed potato production

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## ABSTRACT

**At present, the base for seed potato production is the potato minitubers produced in protected" insect-proof" spaces. For seed potato production it is essential to use a healthy and high-quality biological material, which contributes to obtaining superior production, both quantitatively and qualitatively. In potatoes, to eradicate viruses and obtain a healthy initial material, we have at our disposal the technique of sampling and *"in vitro"* cultivation of meristems. In 2024, 11 potato cultivars grown in greenhouses were evaluated. The mini-tubers are derived from *"in vitro"* cultivated plants. Before micropropagation, the plantlets have been subjected to viral testing using the ELISA technique. Potato microplants used for *"in vitro"* multiplication were obtained from meristem cultures. Regarding the average number of minitubers/pot, the best results were obtained in 'Castrum' (11.50), 'Ervant' (10.80) and 'Sevastia' (10.10) cultivars. The best results in terms of the average weight of minitubers/plant were obtained in 'Braşovia' (271.62 g), 'Darilena' (270.13 g) and 'Ervant' (258.87 g) cultivars. The 'Braşovia' and 'Darilena' cultivars also recorded the highest values of the average weight of minitubers (53.2 g and 50.92 g, respectively). The highest percentage of minitubers >30 mm (60%) was found for 'Braşovia' and 'Darilena' cultivars. Also, for 'Asinaria', 'Cosiana', 'Ervant', 'Foresta', 'Marvis' and 'Sarmis' cultivars, the highest rate was recorded for minitubers in the size fraction 25-30 mm and >30 mm. By selecting appropriate cultivars and managing them according to their specific needs, potato growers can enhance the efficiency of seed production systems and achieve higher yields in field multiplication stages.**

**Keywords**: potato, meristem cultures, micropropagation, minitubers, seed potato production

## INTRODUCTION

High-quality seed is a crucial investment in both low-input and high-input farming systems. The availability of a sufficient supply of healthy tubers is an essential requirement for effective potato production. The quality of seed potato tubers is the most significant factor influencing yield (Bus and Wustman, 2007). Vegetative propagation of potatoes leads to the transmission of viruses from one generation to the next, with virus levels increasing due to repeated propagation (Thomas-Sharma *et al*., 2016; Priegnitz *et al*., 2020). Viral diseases not only increase susceptibility to other pathogens but also lead to economic losses by negatively affecting tuber yield and quality (Lin *et al*., 2014; Adolf *et al*., 2020). Obtaining virus-free plants is necessary for successful viral disease management and sustainable propagation activities, including potato germplasm conservation and global exchange of genetic resources (Naik and Khurana, 2003; Volmer *et al*., 2017; Ellis *et al*., 2020). Because

potatoes are vegetative propagated, viral infections worsen with each successive generation, making the production of healthy material crucial (Faccioli, 2001). Preventive measures can help limit the spread of potato viruses. In seed potato production, a key step is obtaining high-quality phytosanitary initial material. Using superior planting material is fundamental not only for achieving high yields and quality but also for supporting a sustainable production system. While it does not ensure a successful outcome, it provides the crop with a strong start, as the negative impact of using poor-quality initial material cannot be corrected during the growing season (Morrenhof, 1998).

To eliminate viruses and secure healthy initial material in potatoes, we can utilize the technique of sampling and *"in vitro"* cultivation of meristems. Meristems are tissues composed of young cells that retain their ability to proliferate throughout the plant's life. The meristems used for initiating an *"in vitro"* culture are found at the plant's growth tip, in the apical buds, within the leaf axils, in the axillary buds, or the dormant buds of the tubers. Meristem culture serves as the foundation for obtaining healthy material and is the first step in starting *"in vitro"* cultures. This technique enables the intensive propagation of selected individuals with a higher multiplication potential than traditional methods while preserving the genetic uniformity of the biological material. In meristem culture methods, the size of the explant significantly influences the effectiveness of virus eradication. Typically, excising 0.2 mm shoot tips that include the apical dome along with one or two leaf primordia is necessary (Wang *et al*., 2006; Zhang *et al*., 2019). To regenerate the meristematic explant, the apical tip must include at least 1–2 leaf primordia, which are essential for the production of auxins and cytokinins (Bhojwani and Dantu, 2013). The excision of such small shoot tips is a labor-intensive, time-consuming process that requires a high level of skill. Furthermore, the outcomes can vary significantly regarding shoot growth and the effectiveness of virus eradication (Bettoni *et al*., 2016; Magyar-Tábori *et al*., 2021). A notable limitation of these meristem culture-based techniques is the inability to ensure complete removal of viral particles, particularly in cases of mixed infections (Faccioli and Marani, 1998; Zhang *et al*., 2019). The successful elimination of potato viruses relies on both the type of virus targeted for eradication and the size of the meristematic explant being inoculated, which is the imary factor influencing the ability to produce healthy plants. Generally, the success of disease eradication, particularly for viruses, is inversely related to the size of the meristem. Smaller explants tend to have a higher rate of virus elimination, but their regenerative capacity significantly diminishes, resulting in a lower chance of survival for the explant. Typically, the regenerative potential of these meristems is very high. They adapt well to *"in vitro"* conditions, readily recover from the trauma of excision, and resume their activity after being transferred to aseptic media with appropriate nutrient composition and optimal cultivation conditions, ultimately generating plants. This process involves both the growth of the bud axis and its primordia, leading to leaf development and the formation of roots. Ultimately, the terminal, apical, or axial meristem produces plantlets, depending on the hormonal balance present in the culture medium (Cachiţă, 1984).

Due to their high vigor and exceptional health status, *"in vitro"* derived plantlets are essential for producing top-quality, true-to-type, disease-free seed material (Pruski, 2007). One of the most common methods for producing pre-basic seed is cultivating minitubers in greenhouses from *"in vitro"* plantlets obtained from nodal cuttings. These nodal cuttings can be aseptically produced in large quantities by *"in vitro"* laboratories that specialize in rapid multiplication. Minitubers are typically defined as the progeny tubers produced from *"in vitro"* derived plantlets. The term refers to their size, which is smaller than conventional seed tubers but larger than *"in vitro"* tubers (microtubers) produced in aseptic conditions on artificial media. Minituber production has become an integral part of the global seed production system, serving as a link between the rapid *"in vitro"* multiplication using nodal cuttings and the field multiplication of seed tubers (Struik,

2007). The health standard of minitubers is high because they are generated under controlled conditions from aseptically produced *"in vitro"* plantlets. Although minitubers are small, they contain a significant number of eyes, allowing for the potential to produce many sprouts per individual tuber if treated appropriately. However, the vigor of these sprouts will be limited by the resources available from the mother tuber.

## MATERIALS AND METHODS

The potato cultivars used in this study were created at the National Institute of Research and Development for Potato and Sugar Beet, Brasov, Romania. The potato minitubers are produced in a protected *"insect-proof"* space belonging to the Plant Tissue Culture Research Laboratory. Potato microplants regenerated from viable meristematic tissues were subjected to viral testing using the ELISA technique. Healthy clones of each cultivar are then used in the micropropagation process to obtain planting material.
The potato plantlets were transplanted from *"in vitro"* conditions to *"ex vitro"* between May 14-16, 2024. At the beginning of April, the substrate necessary for the production of minitubers was prepared, which consists of a mixture of red peat with bentonite, black peat and perlite (4:2:1). In order for the culture substrate to be richer in nutrients, and complex fertilizer NPK 15:15:15 + 6% sulfur was applied before planting, in the form of granules, after which the pots with the mixture were watered daily (2 hours/day), to facilitate the dissolution of complex granules. During the growing season, preventive treatments against potato late blight (*Phytophthora infestans*) and treatments with foliar fertilizers were applied (Table 1).

**Table 1.** Application schedule for disease control treatments and foliar fertilization during the growing season

| Date of treatment application | Product applied | Dose |
|---|---|---|
| May 27 | Cropmax<br>Razormin<br>Agroleaf Power | 20 ml/10 l water<br>20 ml/10 l water<br>30 g/10 l water |
| June 4th | Cropmax<br>Razormin<br>Agroleaf Power | 20 ml/10 l water<br>20 ml/10 l water<br>30 g/10 l water |
| June 10 | Cropmax<br>Zetanil | 20 ml/10 l water<br>0.45 kg/ha |
| June 21 | Cropmax<br>Cimbal | 20 ml/10 l water<br>0.25 kg/ha |
| July 5th | Carial<br>Cropmax<br>Agroleaf Power | 0.6 l/ha<br>20 ml/10 l water<br>30 g/10 l water |
| July 19 | Banjo<br>Cropmax<br>Agroleaf Power | 0.3 l/ha<br>20 ml/10 l water<br>30 g/10 l water |
| August 2 | Zetanil | 0.45 kg/ha |
| August 14 | Infinito | 1.4 l/ha |
| August 30 | Shirlan | 0.3 l/ha |

The pots used for transplanting are made of plastic with a diameter of 17 cm, a height of 12.9 cm and a volume of 2 l (manufacturer Desch Plantpak BV, Netherlands). During the vegetation period (Figure 1), to ensure the nutrients necessary for the growth and development of potato plants, foliar fertilizers treatments were applied. Their activity is based on the combination of microelements, amino acids, vitamins and polysaccharides.

The application of these products ensures a rapid development of the root system and activates the development of the leaf mass, helping the plants to realize their biological potential.

**Figure 1**. Potato plants obtained from *"in vitro"* cultures, in vegetation

Starting from August 30, the plants were no longer watered, and after 2 weeks after the interruption of watering, the haulms were removed. This activity of removing the potato stems helps to suberization of minitubers skin, and this aspect is important for a proper preservation of the biological material in the warehouse.
The potato minitubers were harvested between October 1-8 (Figure 2). After harvesting, minitubers of the 11 potato cultivars were evaluated in terms of the following parameters: average number of minitubers/pot, the average weight of minitubers/pot, the average weight of minitubers. The calibration of the minitubers by size classes was also carried out.

**Figure 2.** Aspects from the harvesting of minitubers

## RESULTS AND DISCUSSIONS

In this study, 11 potato cultivars were grown in protected areas in order to obtain a high-quality pre-basic seed from *"in vitro"* plantlets. After 19 weeks from planting, the minitubers were harvested and evaluated in terms of number, weight, and size.

### The influence of the cultivars on the number of minitubers/plant

The number of *"in vitro"* plantlets typically range from 2 to 5, but this can vary significantly depending on the specific cultivar and how the crop is managed. The number of minitubers per individual *"in vitro"* microplants is an important yield component. The results presented in Figure 3 highlight the fact that the highest number of minitubers/plant was obtained in 'Castrum' (11.50), 'Ervant' (10.80) and 'Sevastia' (10.10) cultivars. This aspect has a major importance on the production capacity of the cultivars. If the potato plants produce more minitubers, the productivity of cultivars is higher.
The lowest values, in terms of the average number of minitubers/plant, were obtained in the 'Cosiana' (4.7), 'Brașovia' (5.3) and 'Darilena' (5.6) cultivars.

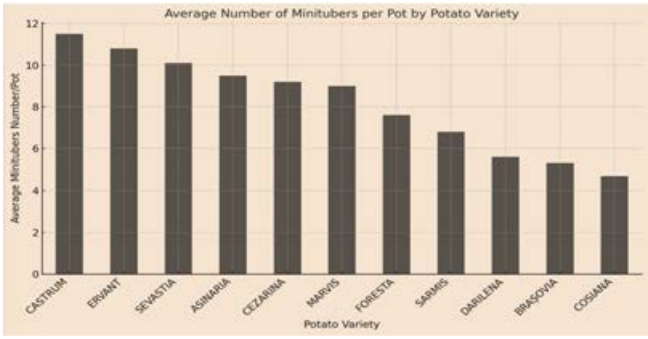



**Figure 3.** Effect of potato cultivars on the average number of minitubers/plant

**The influence of the cultivar on the average weight of minitubers/plant**

The Figure 4 shows the evolution of average weight of minituber depending on the analyzed potato cultivars.

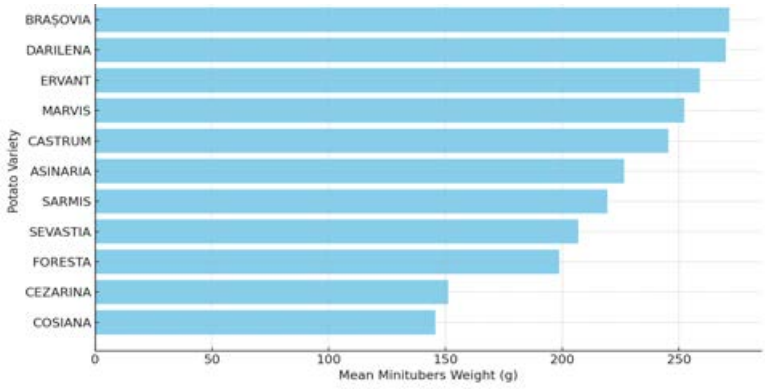



**Figure 4.** Comparison of mean minituber weight/plant across potato cultivars

The best results in terms of the average weight of minitubers/plant were obtained in Braşovia (271.62 g), Darilena (270.13 g) and Ervant (258.87 g).

The small number of minituberc recorded in the 'Braşovia' and 'Darilena' cultivars. (Figure 3) attracted their greater weight (Figure 4). It should be noted that the number of minitubers (Figure 3) did not influence their weight (Figure 4) for the 'Ervant' *cv.* This cultivar recorded a number of minitubers of 10.80 (Figure 3) and an average weight of 258.87 g (Figure 4).

**Minitubes weighing less than 60 g, depending on the cultivars**

The cultivar plays a critical role in determining the average weight of minitubers, which directly impacts the overall production efficiency. Different cultivars have unique genetic traits that influence tuber size, growth patterns, and yield potential. These genetic characteristics are essential for understanding how a particular cultivar will perform under specific conditions and how it contributes to the final output.

Figure 5 shows the results regarding the average weight of minitubers for the 11 evaluated potato cultivars. For this analyzed parameter, the 'Braşovia' and 'Darilena' *cvs.* stood out with an average weight of the minitubers of 53.20 g and 50.92 g, respectively. The two cultivars obtained the highest values also in the case of the average weight of minitubers/plant.

The lowest values were obtained for the 'Cezarina' (20.14 g) and 'Sevastia' (20.84 g) cultivars.

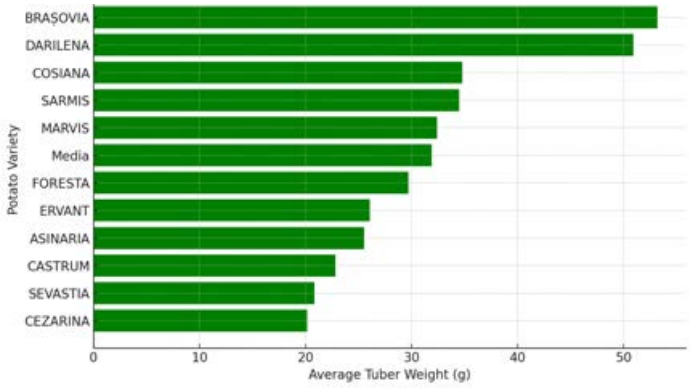
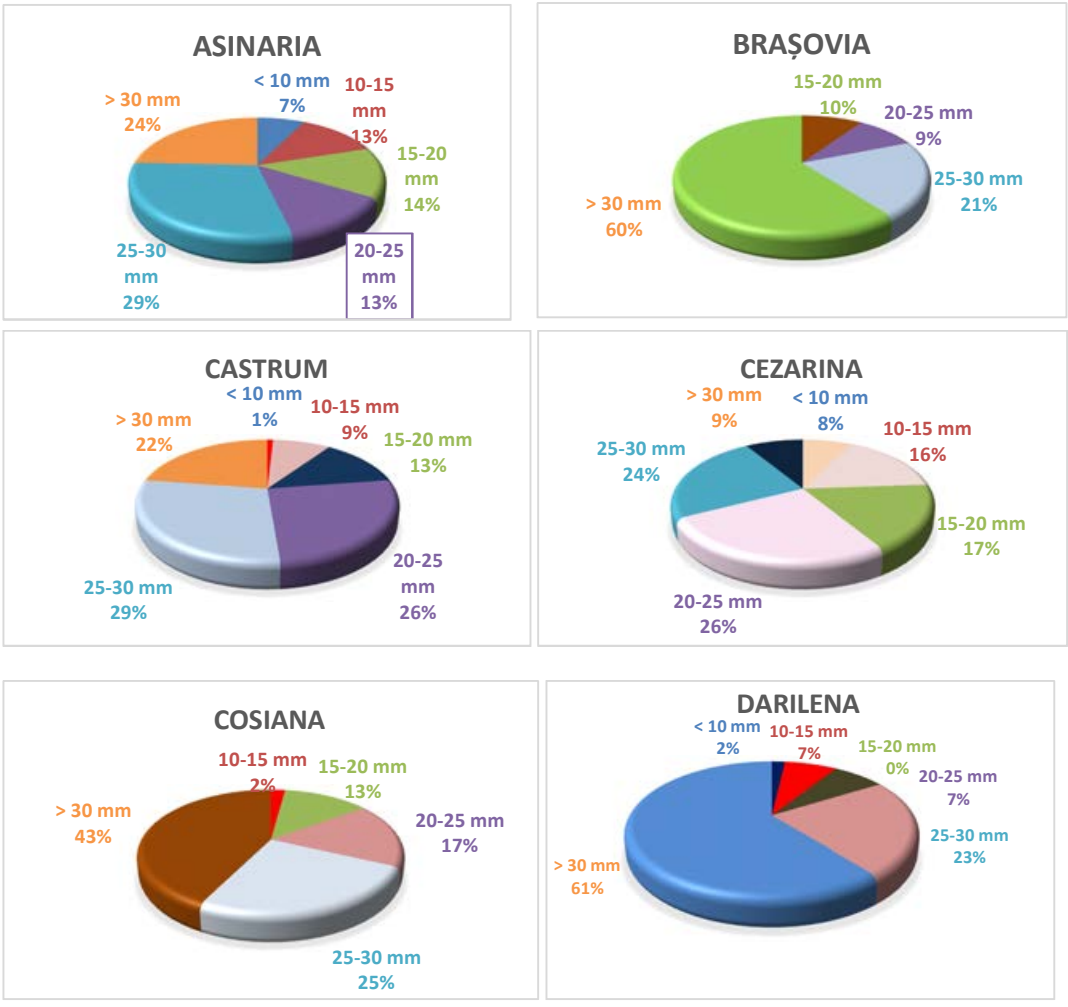



**Figure 5.** Average minituber weight of potato cultivars

**Minitubers calibration by size classes**

The size of the minituber plays a crucial role, as it influences the dormancy period, the vigor of the seed tuber, the number of stems that can be successfully generated, the speed of emergence, the survival rate of plants and stems, and the overall yield potential. Although minitubers are small, they still contain a significant number of eyes and, with proper treatment, can generate numerous sprouts from each tuber.

Figure 6 shows the percentage distribution of mini tubers according to the calibration classes and the percentages.



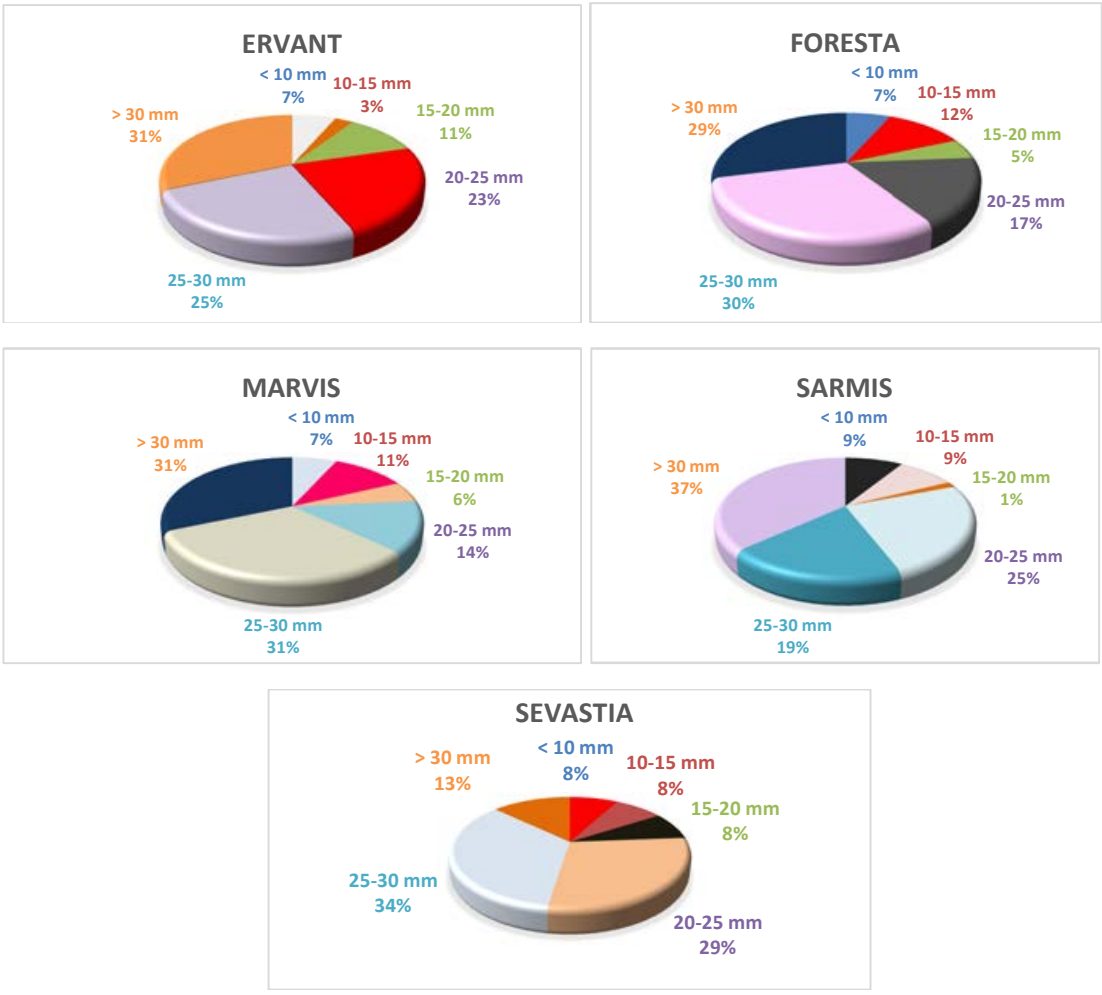



**Figure 6.** Percentage distribution of minitubers on calibration classes according to cultivar

Among the 11 potato cultivars evaluated, in 8 of them ('Asinaria', 'Braşovia', 'Cosiana', 'Darilena', ' Ervant', 'Foresta', 'Marvis' and 'Sarmis'), following the percentage distribution of minitubers by calibration classes, it can be seen that the minitubers were mainly in the size fraction 25-30 mm and >30 mm. For 'Braşovia' and 'Darilena' *cvs.*, the percentage of >30 mm minitubers was 60% and 61%, respectively.
For 'Castrum', 'Cezarina', and 'Sevastia' *cvs.*, the harvested minitubers mainly fell into the 20-25 mm and 25-30 mm size classes (24-34%).

**CONCLUSIONS**

This study highlights the diverse potential of potato cultivars in minituber production. Generally, larger minitubers contain more stored nutrients, which promotes early growth, quicker emergence, and overall healthier plants. This increased vigor contributes to stronger stem and root development, enabling better nutrient and water uptake, and potentially boosting yields. Conversely, cultivars that naturally produce smaller minitubers can still perform well but may require more careful management to optimize their growth.
The use of *"in vitro"* multiplication technologies ensures the production of biologically healthy material from genotypes suited to sustainable agriculture, yielding clonal material that is from a higher phytosanitary category. By selecting the appropriate cultivars and

tailoring their management to specific needs, potato growers can enhance seed production system efficiency and improve yields during field multiplication stages.

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# Cabbage breeding tools for biotic and abiotic resistance

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## ABSTRACT

**Cabbage (*Brassica oleracea* var. *capitata*) is an important vegetable crop grown globally for its nutritional value and economic importance. However, cabbage production faces significant challenges from various biotic and abiotic stresses, including pests, diseases, and environmental factors such as drought, heat, and salinity. Developing cabbage cultivars with improved resistance to these stresses is crucial for sustainable and productive agriculture. This review article examines the latest breeding tools and approaches used to enhance biotic and abiotic stress resistance in cabbage. It explores traditional breeding methods, marker-assisted selection, genetic engineering, genome editing techniques like CRISPR/Cas9, and emerging technologies such as genomic selection and speed breeding. Furthermore, the article discusses the integration of -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, to accelerate the development of stress-resistant cabbage cultivars. The study also highlights the importance of incorporating farmer preferences and participatory breeding strategies to ensure the adoption and success of these improved cabbage cultivars.**

**Keywords***:* crop improvement, cole crops, breeding tools, stress tolerance, breeding approaches.

## INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata*) is a cool-season vegetable crop belonging to the Brassicaceae family. It is widely cultivated and consumed around the world for its nutritional value, as it is a rich source of vitamins, minerals, and fiber (Sarıkamış *et. Al*, 2009). Cabbage production plays a significant role in the global economy, with an annual production of over 70 million tons (http://www.fao.org/faostat/en/#data/QC). However, cabbage cultivation faces various biotic and abiotic stresses that can significantly reduce yield and quality. Biotic stresses, such as insect pests, fungal diseases, bacterial diseases, and viral diseases, pose major threats to cabbage production (Juroszek, P., and Tsai, 2009). Some of the most common insect pests affecting cabbage include cabbage loopers, cabbage root flies, cabbage aphids, and diamondback moths (Shelton, A. M., and Badenes-Perez, 2006). Fungal diseases like clubroot, black rot, and downy mildew can cause severe damage to cabbage crops (Rimmer *et. al.,* 2007). Bacterial diseases, such as black rot and bacterial soft rot, and viral diseases like cauliflower mosaic virus and turnip mosaic virus, also contribute to yield losses (. Bhat, K. A., and Kolanjakkaren, 2014; Shukla and Tenzer, 2017). Abiotic stresses, including drought, heat, salinity, and nutrient deficiencies, can also

have detrimental effects on cabbage growth, development, and productivity (Farooq *et. al*., 2014). Climate change is exacerbating these abiotic stresses, making it increasingly challenging for farmers to maintain stable cabbage yields (Fahad *et. al.,* 2017). Developing cabbage cultivars with improved resistance to biotic and abiotic stresses is crucial for sustainable and productive agriculture.

**TRADITIONAL BREEDING METHODS**

Traditional breeding methods have been extensively used in cabbage improvement programs for decades. These methods involve selecting and crossing parent lines with desirable traits, followed by multiple generations of selfing and selection to develop improved cultivars (Bradshaw, 2016).

**Pedigree breeding:** Pedigree breeding is a commonly used method in cabbage breeding programs. It involves selecting superior individuals from segregating populations and self-pollinating them for several generations to develop pure lines. These pure lines can then be crossed to combine desirable traits, such as pest resistance or improved yield (Prakas *et. al.,* 2014).

**Recurrent selection:** Recurrent selection is another traditional breeding approach used in cabbage breeding. It involves cyclically selecting and intermating superior individuals from a population to gradually increase the frequency of favourable alleles for target traits, such as disease resistance or stress tolerance (. Shukla and Sundaram,2004).

**Hybridization and heterosis breeding:** Hybridization involves crossing two genetically diverse parent lines to produce $F_1$ hybrids that exhibit heterosis or hybrid vigour. Heterosis breeding in cabbage has been successful in developing high-yielding and stress-tolerant hybrids by exploiting the phenomenon of hybrid vigour (Shukla, S., Naik, A. K., and Sundaram , 2014).

**Mutation breeding:** Mutation breeding involves inducing genetic variations through physical or chemical mutagens, such as gamma rays, X-rays, or ethyl methane sulfonate (EMS). These mutations can lead to new trait variations, including enhanced biotic or abiotic stress resistance, which can be selected and incorporated into breeding programs ( Ahloowalia et.al., 2004).

While traditional breeding methods have contributed significantly to cabbage improvement, they have limitations, such as long breeding cycles, the polygenic nature of many traits, and the difficulty in pyramiding multiple resistance genes (Tester, and Langridge, 20010). To overcome these challenges, modern breeding approaches, including marker-assisted selection, genetic engineering, and genome editing, have been developed and integrated into cabbage breeding programs.

**MARKER-ASSISTED SELECTION (MAS)**

Marker-assisted selection (MAS) is a breeding approach that utilizes molecular markers linked to specific traits or genes of interest to facilitate the selection of desirable genotypes (Collard, and Mackill, 2008), MAS has been widely applied in cabbage breeding for various traits, including disease resistance, insect resistance, and abiotic stress tolerance.

**Resistance gene mapping and MAS:** Molecular markers have been developed and used for marker-assisted selection of resistance genes against various cabbage diseases, such as black rot, fusarium wilt, and clubroot (Sharma, *et. al.,* 2017; Ren et. al., 2001; Chandra *et. al.,* 20211). For example, the Crr1 gene conferring resistance to clubroot disease has been

mapped and validated, enabling the development of molecular markers for MAS in cabbage breeding programs (Hirai, 2006).

**QTL mapping and MAS for complex traits:** Quantitative trait loci (QTL) mapping has been employed to identify genomic regions associated with complex traits, such as yield, quality, and abiotic stress tolerance in cabbage. Molecular markers linked to these QTLs have been used for marker-assisted selection to improve these traits (Lee *et. al.,* 2021; Zhang *et. al.,* 2020).

**Genomic selection (GS):** Genomic selection is an advanced form of MAS that utilizes genome-wide marker data and statistical models to predict the breeding values of individuals for complex traits (Meuwissen et. al., 2001). GS has shown promising results in cabbage breeding for traits like drought tolerance, where it has been used to accelerate the breeding cycle and improve selection accuracy (Bhandari *et. al.,* 2022).

While MAS has facilitated more efficient and precise selection in cabbage breeding, it has limitations, such as the need for extensive marker development and validation, and the potential for linkage drag (Bernardo *et. al.,* 2008). Additionally, MAS may not be effective for traits controlled by many minor-effect QTLs or epistatic interactions.

**GENETIC ENGINEERING**

Genetic engineering involves the direct transfer and integration of specific genes from various sources into the cabbage genome to introduce desirable traits (Bawa, and Anilakumar, 2013). This approach has been explored in cabbage breeding for enhancing biotic and abiotic stress resistance.

**Insect resistance:** Genes encoding insecticidal proteins, such as Bacillus thuringiensis (Bt) toxins, have been introduced into cabbage to confer resistance against lepidopteran pests like cabbage loopers and diamondback moths (Cao et. al., 2002). These *Bt* cabbage lines have shown improved protection against insect damage and reduced the need for insecticide applications.

**Disease resistance:** Transgenic approaches have been used to develop cabbage cultivars with resistance against various fungal, bacterial, and viral diseases. For example, the introduction of antifungal proteins, like chitinases and glucanases, has been explored for enhancing resistance against fungal pathogens like clubroot and downy mildew (Muzzarelli, *et. al.,* 2001; Mora, and Earle, 2001).

**Abiotic stress tolerance:** Genetic engineering has also been employed to improve abiotic stress tolerance in cabbage. Genes involved in stress response pathways, such as those encoding transcription factors, Osmo protectants, or antioxidant enzymes, have been introduced into cabbage to enhance drought, heat, and salinity tolerance (Wang, *et. al.,* 2003; Bajji *et. al.,* 2001).

While genetic engineering has shown promising results in cabbage improvement, concerns over the potential risks associated with genetically modified (GM) crops have led to strict regulations and public acceptance challenges in some regions (Nicolia, *et.al.,* 2014). Additionally, the complexity of many stress tolerance mechanisms and potential unintended effects remains challenges in genetic engineering approaches.

**GENOME EDITING**

Genome editing technologies, particularly CRISPR/Cas9, have emerged as powerful tools for precise and targeted modifications of the cabbage genome (Boetesi and Fischer, 2015).

These techniques offer advantages over traditional genetic engineering by enabling site-specific modifications without the introduction of foreign DNA.

**Disease resistance:** CRISPR/Cas9 has been used to knockout or modify susceptibility genes in cabbage to enhance resistance against various diseases. For example, editing the BraA.FR.a gene, which encodes a susceptibility factor for the Fusarium oxysporum fungus, has been shown to confer resistance against Fusarium wilt disease in cabbage (Chaturvedi *et. al.,* 2012).

**Insect resistance:** CRISPR/Cas9 has also been employed to modify endogenous cabbage genes involved in defence pathways or plant-insect interactions to enhance insect resistance. For instance, editing the BrPDF2.1 gene, which encodes a plant defensin protein, has been reported to improve resistance against diamondback moths (Hu *et. al.,* 2019).

**Abiotic stress tolerance:** Genome editing has been explored for improving abiotic stress tolerance in cabbage by modifying genes involved in stress response pathways. For example, editing the BrDREB2A transcription factor gene has been shown to enhance drought and heat tolerance in cabbage (Sakuraba*, et. al*., 2017).

While genome editing offers promising opportunities for cabbage improvement, there are challenges associated with off-target effects, regulatory frameworks, and public acceptance, similar to those faced by genetic engineering (Lassoued *et. al.,* 2020). Additionally, the complex nature of many stress tolerance mechanisms may require multiplex editing of multiple genes or regulatory elements.

**EMERGING TECHNOLOGIES**

In addition to the established breeding tools mentioned above, several emerging technologies are being explored and integrated into cabbage breeding programs for enhancing biotic and abiotic stress resistance.

**Speed breeding:** Speed breeding is a novel technique that involves the use of controlled environmental conditions, such as extended photoperiods and optimized temperature and humidity, to accelerate the breeding cycle (Watson *et. al.,* 2018). By shortening the generation time, speed breeding can significantly reduce the time required for developing improved cabbage cultivars with desired stress resistance traits.

**Genomic selection (GS):** As mentioned earlier, genomic selection is an advanced form of marker-assisted selection that utilizes genome-wide marker data and statistical models to predict breeding values for complex traits (Meuwissen *et. al.,* 2001). GS has shown promising results in cabbage breeding for traits like drought tolerance and yield, and its application is expected to grow as genotyping costs decrease and computational power increases (Bhandari *et. al.,* 2022).

**Epigenetic breeding:** Epigenetic modifications, such as DNA methylation and histone modifications, can influence gene expression and phenotypic variation without altering the underlying DNA sequence (Baulcombe, and Dean, 2014). Epigenetic breeding involves exploiting these modifications to modulate stress response pathways and improve stress tolerance in cabbage. This approach has shown potential in enhancing drought and salt tolerance in crops like Brassica napus (Hauben et. al., 2009).

**Synthetic biology and metabolic engineering:** Synthetic biology and metabolic engineering approaches involve the design and construction of novel genetic circuits or metabolic pathways to enhance specific traits in plants (Liu, and Stewart, 2015). These techniques have been applied in model plants like Arabidopsis thaliana for improving stress tolerance and could be explored in cabbage for enhancing biotic and abiotic stress resistance.

**INTEGRATION OF-OMICS APPROACHES**

The integration of various -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, has provided valuable insights into the molecular mechanisms underlying biotic and abiotic stress responses in cabbage. These insights can inform and accelerate breeding efforts for developing stress-resistant cultivars.

**Genomics:** Advances in next-generation sequencing technologies have enabled the generation of high-quality reference genomes for cabbage and its close relatives (Liu, et. al., 2014). Comparative genomic analyses have identified genes and regulatory elements associated with stress response pathways, which can serve as targets for breeding or genome editing strategies.

**Transcriptomics:** Transcriptomic studies, using techniques like RNA-seq, have been employed to investigate gene expression patterns in cabbage under various biotic and abiotic stress conditions (Yang *et. al.,* 2021[a]; Yang *et. al*., 2010[b]). These analyses have identified stress-responsive genes, transcription factors, and regulatory networks, which can be targeted for improving stress tolerance through breeding or genetic engineering.

**Proteomics:** Proteomic approaches, such as two-dimensional gel electrophoresis and mass spectrometry, have been used to study changes in protein abundance and post-translational modifications in cabbage under stress conditions (*Gao et. al*., 2009; Peng *et. al*., 2009). These analyses have identified stress-responsive proteins and protein complexes involved in defence mechanisms, which can be targeted for enhancing stress resistance.

**Metabolomics:** Metabolomic studies have investigated changes in the metabolite profiles of cabbage under various biotic and abiotic stresses (Savchenko *et. al.,* 2010; Martínez-Ballesta, *et al*., 2013). T

hese analyses have identified stress-responsive metabolites and metabolic pathways that can be modulated through breeding or metabolic engineering to improve stress tolerance.

By integrating information from these -omics approaches, researchers can gain a comprehensive understanding of the complex molecular networks underlying stress responses in cabbage.

This knowledge can guide the identification of key target genes, proteins, or metabolic pathways for manipulation through breeding, genetic engineering, or genome editing strategies to develop stress-resistant cabbage cultivars.

**PARTICIPATORY AND FARMER-CENTRIC BREEDING**

While technological advancements in breeding tools and -omics approaches are crucial for developing stress-resistant cabbage cultivars, it is equally important to consider farmer preferences and involve them in the breeding process. Participatory and farmer-centric breeding approaches have gained increasing recognition for ensuring the adoption and success of improved cultivars.

**Participatory plant breeding (PPB):** PPB involves the active participation of farmers in the breeding process, from setting breeding goals and selecting parent materials to evaluating and selecting advanced lines (Ceccarelli, 2015). This approach ensures that the developed cultivars meet the specific needs and preferences of farmers, increasing the likelihood of adoption and successful cultivation.

**Farmer-led evaluation and selection:** Farmer-led evaluation and selection involve farmers directly assessing and selecting promising cabbage lines under their local environmental conditions and management practices (Buckler *et. al*., 2021). This approach ensures that the selected lines are well-adapted to the target production environments and farmer preferences.

**Incorporation of farmers' traditional knowledge:** Traditional knowledge held by farmers can provide valuable insights into local adaptation strategies, stress tolerance mechanisms, and desirable traits (Altieri, 2014). Incorporating this knowledge into breeding programs can enhance the development of stress-resistant cabbage cultivars that are better suited to local conditions and cultural preferences.

**Community seed banks and seed exchange networks:** Community seed banks and seed exchange networks facilitate the conservation, exchange, and distribution of locally adapted cabbage cultivars (Vornooy *et. al*., 2015).

These initiatives promote the maintenance of genetic diversity and ensure the availability of stress-tolerant cultivars to farmers, particularly in marginalized communities.
By involving farmers and considering their preferences and local knowledge, breeding programs can develop stress-resistant cabbage cultivars that are not only high-yielding and resilient but also meet the specific needs and cultural preferences of farmers, increasing the likelihood of successful adoption and sustainable production.

**Table 1.** Major insect pests of cabbage and their management strategies

| Insect Pest | Damage Caused | Management Strategies |
|---|---|---|
| Cabbage Looper | Defoliation | Bt crops, Biological control, Insecticides |
| Diamondback Moth | Defoliation | Bt crops, Trap crops, Resistance breeding |
| Cabbage Root Fly | Root damage | Crop rotation, Insecticides, Resistant cultivars |
| Cabbage Aphid | Stunting, Virus transmission | Insecticides, Biological control, Resistant v cultivars |

**Table 2.** Common fungal diseases of cabbage and their management

| Disease | Causal Pathogen | Management Strategies |
|---|---|---|
| Clubroot | *Plasmodiophora brassicae* | Resistant cultivars, Crop rotation, Soil amendments |
| Black Rot | *Xanthomonas campestris* pv. *campestris* | Resistant cultivars , Seed treatment, Crop rotation |
| Downy Mildew | *Peronospora parasitica* | Fungicides, Resistant cultivars, Cultural practices |
| Alternaria Leaf Spot | *Alternaria brassicae* | Fungicides, Resistant cultivarss, Cultural practices |

**Table 3.** Examples of biotic stress resistance genes used in cabbage breeding

| Gene | Source | Trait | Breeding Approach |
|---|---|---|---|
| Bt Cry genes | *Bacillus thuringiensis* | Insect resistance | Genetic engineering |
| Crr1 | *Brassica rapa* | Clubroot resistance | Marker-assisted selection |
| BjuPR-1, BjuPR-4, BjuDF1.2 | *Brassica juncea* | Fungal disease resistance | Marker-assisted selection |
| BraA.FR.a (knockout) | *Brassica rapa* | Fusarium wilt resistance | CRISPR/Cas9 genome editing |

**Table 4.** Examples of abiotic stress tolerance genes used in cabbage breeding

| Gene | Function | Trait | Breeding Approach |
|---|---|---|---|
| BrDREB2A | Transcription factor | Drought, Heat tolerance | CRISPR/Cas9 genome editing |
| Osmoprotectant genes | Osmolyte biosynthesis | Drought, Salinity tolerance | Genetic engineering |
| Antioxidant enzyme genes | ROS scavenging | Drought, Heat tolerance | Genetic engineering |
| Aquaporin genes | Water transport | Drought tolerance | Marker-assisted selection |

**Table 5.** Examples of QTLs and genomic regions associated with abiotic stress tolerance in cabbage

| Trait | QTL/Genomic Region | Mapping Population |
|---|---|---|
| Drought tolerance | QTLs on C02, C05, C09 | DH population |
| Cold tolerance | QTLs on C03, C05, C08 | F2:3 population |
| Yield under drought | Genomic regions on C02, C06 | RIL population |

## CONCLUSIONS

This article has explored various breeding tools and approaches used to enhance biotic and abiotic stress resistance in cabbage.

Traditional breeding methods, marker-assisted selection, genetic engineering, and genome editing have all contributed to the development of improved cabbage cultivars with enhanced resistance to pests, diseases, and environmental stresses.

Emerging technologies, such as speed breeding, genomic selection, epigenetic breeding, and synthetic biology, offer promising opportunities for further advancements in this field.

The integration of -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, has provided valuable insights into the molecular mechanisms underlying stress responses in cabbage.

This knowledge can guide the identification of key target genes, proteins, or metabolic pathways for manipulation through breeding or genetic engineering strategies to develop stress-resistant cultivars.

However, the successful adoption and impact of these improved cabbage cultivars also depend on considering farmer preferences and involving them in the breeding process through participatory and farmer-centric approaches.

By incorporating traditional knowledge, facilitating farmer-led evaluations, and promoting community seed banks and seed exchange networks, breeding programs can develop stress-resistant cabbage cultivars that meet the specific needs and cultural preferences of farmers, ensuring sustainable and productive agriculture.

As climate change and global population growth continue to exert pressure on agricultural systems, the development of stress-resistant cabbage cultivars will become increasingly crucial for ensuring food security and promoting sustainable agriculture.
The integration of diverse breeding tools, -omics approaches, and farmer-centric strategies will play a vital role in achieving this goal and contributing to the resilience of cabbage production systems worldwide.

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