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Seed germination and biomass of cabbage (*Brassica oleracea* var. *capitata*) seedlings in conventional agriculture (ConvAg) and cold-water agriculture (ColdAg)

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ABSTRACT

Objective: To evaluate the effects of cold-water agriculture (ColdAg) *vs.* conventional agriculture (ConvdAg) conditions on seed germination and fresh and dry biomass accumulation of cabbage (*Brassica oleracea* var. *capitata* L.) seedlings.

Design/methodology/approach: The effects on germination parameters and fresh and dry biomass accumulation of cabbage (*Brassica oleracea* var. *capitata* L.) seedlings were evaluated under the following treatments: (1) cold-water agriculture conditions (ColdAg: 35 °C temperature inside the growth chamber, 14.8 °C water flow temperature, 85% relative humidity) and (2) conventional agriculture (ConvAg: 25 °C temperature inside the growth chamber, 20 °C irrigation water temperature, 70% relative humidity). In both cases, the light intensity was 334 μmol m^{-2} s^{-1}. Each condition had three replicates, and the experimental unit was a pot with commercial substrate in which 10 seeds were sown. The experiment was conducted in controlled growth chambers, and the treatments were distributed in these completely at random. The germination data obtained were used to estimate the variables daily total germination (DTG), median germination time (T_{50}), germination percentage (GP), coefficient of velocity of germination (CVG), and germination index (GI). In addition, the weight of the fresh and dry biomass of the seedlings was determined. With the results obtained, variance analysis and Tukey mean comparison tests were performed ($p \leq 0.05$).

Results: Seed germination in ConvAg occurred 2 d earlier than in ColdAg. Likewise, daily total germination (DTG) was higher in ConvAg. However, fresh and dry seedling biomass was higher in ColdAg, exceeding the ConvAg treatment by 66.3 and 146.7%, respectively.

Limitations of the study/implications: This technology requires sufficient space and specialized technical and scientific equipment, as well as the availability of cold water throughout the crop cycle.

Findings/conclusions: ColdAg technology allowed good germination of cabbage seeds and greater production of fresh and dry seedling biomass.

Keywords: Brassicaceae, cabbage, ColdAg, ConvAg, germination, biomass.

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INTRODUCTION

Global food security faces serious challenges related to the impacts of global climate change on agriculture and the increasing demand for food by the ever-growing population. This makes it necessary to develop new technologies that allow food production under limiting conditions (FAO, 2017), which in turn are energy efficient and environmentally sustainable in the long term (Rabbi *et al.*, 2019; WMO, 2023; García-Huante *et al.*, 2024; Xiao *et al.*, 2024). These include cold-water agriculture (ColdAg) or conditioning for soil cooling in areas under controlled warm climate conditions, where thermal stress is generated in the roots of the crop, obtaining plants with better development and growth (Graamans *et al.*, 2018). This technology was developed from a marine renewable energy system known as Ocean Thermal Energy Conversion (OTEC), through which cold deep seawater and warm surface water are pumped to produce electrical energy, and the volume of ocean water can be used to generate temperate zone crops in tropical areas (García-Huante *et al.*, 2024). Under these conditions, higher yields per unit area have been reported, as well as greater efficiency in water use, compared to crops under conventional agriculture (ConvAg) conditions. This technology is being implemented in some Asian countries with densely populated areas and where the consumption of cabbage, lettuce, tomato, and radish is high (Ghosh and Ganguly, 2017). In this study, cabbage was used as a model crop, a species that can adapt to a wide range of environmental conditions. It is a vitamin C-rich species with high demand in the fresh market, as it has an optimal shelf life for long-distance shipments and high yield. The objective of the study was to compare the effects of conventional agriculture (ConvAg) and cold-water agriculture (ColdAg) conditions on germination variables and fresh and dry biomass production.

MATERIALS AND METHODS

Biological material and experiment setup

Cabbage (*Brassica oleracea* var. *capitata* L.) cv. Royal Vantage (Sakata Seed America, Morgan Hill, CA, USA) seeds were used. This research was conducted under the conditions described in Table 1. Under both experimental conditions, the light intensity was 334 μmol m^{-2} s^{-1}.

For each treatment, three 300 mL plastic pots (height 7.5 cm, base 7 cm, mouth 10 cm) were used. The pots were filled with the commercial substrate. The physical and chemical properties of the substrate are described in Table 2.

Table 1. Experimental conditions to test the effects of cold-water agriculture (ColdAg) on germination and early growth of cabbage (*Brassica oleracea* var. *capitata*) compared to conventional agriculture (ConvAg).

Treatment	Temperature	Relative humidity (%)	Total seeds per pots	Total seeds
ColdAg	35 °C in germination chamber/Cold water flow at 14.8 °C	85	10	30
ConvAg	25 °C in germination chamber/ Irrigation water at 20 °C	70	10	30

Table 2. Chemical and physical properties of the commercial substrate used to test the effects of conventional agriculture (ConvAg) and cold-water agriculture (ColdAg) on seed germination and early seedling growth of cabbage (*Brassica oleracea* var. *capitata*).

pH	**Electrical conductivity ($dS\ m^{-1}$)**	**Bulk density ($g\ cm^{-3}$)**		**Ca**	**K**	**Mg**	**Na**
				($cmol_{(+)}\ kg^{-1}$)			
6.0	0.402	0.658		18.264	9.278	12.807	3.015
Cu	**Fe**	**Mn**	**Zn**	**Field capacity**		**Permanent wilting point**	
($mg\ kg^{-1}$)				**(% gravimetric water content)**			
1.595	30.525	64.568	34.024	73		55	
Porosity (%)							
Total			**Airing**		**Water retention**		
68			22		46		
Unavailable water		**Easily available water**		**Reserve water**		**Hardly available water**	
(%)							
10		6		7		32	

Thirty seeds per treatment were sown in three pots (10 seeds per pot, experimental unit) at a depth of 0.5 cm in a horizontal arrangement.

For ColdAg conditions, a 0.9 mm diameter plastic hose was passed horizontally at the middle height of each of the three pots through which cold distilled water (14.8 °C) was constantly recirculated in a closed-circuit system. The cold water was moved by means of a water chiller (S&A CW-3000, Guangzhou, China), which integrates an internal hydrostatic pump, a metal coil and a fan, in order to cool the substrate placed in the pots (Figure 1, upper image). The experimental units of the ColdAg treatment were placed in the upper part of a growth chamber (Shellab® LI15, Cornelius, OR, USA), on a plastic tray. The incubation conditions were: day and night temperatures of 35 and 30 °C, respectively; 12 h of light; relative humidity of 85%; and light intensity of 334 μmol m^{-2} s^{-1}.

For ConvAg (Figure 1, bottom image), a second growth chamber was used where the experimental units were placed. The conditions inside the chamber were: day and night temperatures of 25 and 20 °C, respectively; 12 h of light; relative humidity of 70%; and light intensity of 334 μmol m^{-2} s^{-1}. In this treatment, there was no cold-water flow to lower the temperature of the substrate in the root zone.

In both treatments (ColdAg and ConvAg), each pot was initially watered with 100 mL of tap water, then the substrate was watered until reaching field capacity twice a day (at 08:00 h and at 16:00 h).

The experiment lasted 23 d from sowing the seeds to harvesting the seedlings.

Germination

Germinated seeds were recorded every 24 h for 16 d. The following variables were estimated from these data: median germination time (T_{50}), germination percentage (GP), daily total germination (DTG), coefficient of velocity of germination (CVG), and germination index (GI), according to the methodologies described by González-Zertuche and Orozco-Segovia (1996) and Kader (2005) (Table 3).

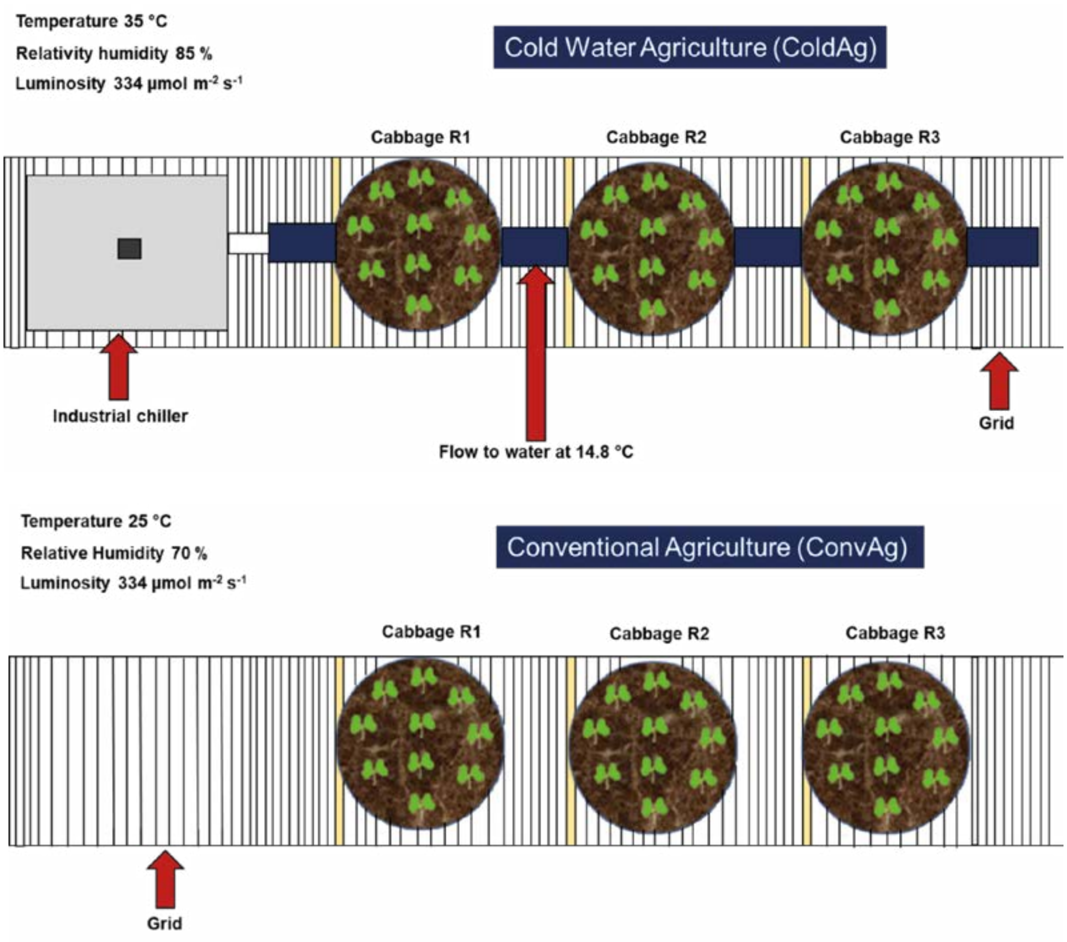

Figure 1. Schematic representation of seeds and plant distribution of cabbage (*Brassica oleracea* var. *capitata*) in the growth chamber to test the effects of cold agriculture (ColdAg, upper image) in comparison with conventional agriculture (ConvAg, bottom image).

Table 3. Germination parameters, formulas and description of variables considered to evaluate the effects of cold-water agriculture (ColdAg) on the germination of cabbage (*Brassica oleracea* var. *capitata*) seeds compared to conventional agriculture (ConvAg).

Parameter	Formula	Description
Median germination time (T_{50})	*Time in days in which* 50% *germination is obtained*	The value of the average germination time implies the number of days necessary to have 50% of the sown seeds germinated.
Germination percentage (GP)	$GP = No.\ of\ seeds\ germinated\ in\ a\ seed\ lot \times 100$	The higher the GP value, the greater the germination of a seed population.
Daily total germination (DTG)	$DTG = No.\ of\ seeds\ germinated\ daily$	It is the value of germinated seeds per day until the end of the germination process.
Coefficient of velocity of germination (CVG)	$CVG = \frac{Total\ of\ seeds\ germinated}{\sum(N1 + N2/2 + ...Nn/n)}$	The CVG gives an indication of the rapidity of germination. It increases when the number of germinated seeds increases and the time required for germination decreases. Theoretically, the highest CVG possible is 100. This would occur if all seeds germinated on the first day.
Germination index (GI)	$GI(\%) = RSG - RGR/100$	Indicate the relation of the relative seed germination (RSG) and the relative growth of radicle (RGR).

Fresh and dry biomass

At 23 d after sowing, the seedlings were harvested. The weight of the fresh biomass was obtained from each replicate, and after drying in a forced air oven at 72 °C for 72 h, the weight of the dry biomass was obtained. In both cases, an analytical scale was used (Adventurer Pro AV213C®, Ohaus; Parsippany, NJ, USA).

Statistical analysis

A variance analysis was performed on the data obtained and the means were compared with the Tukey test with a significance value of 95%. For these analyses, the statistical software SAS version 9.4 was used.

RESULTS AND DISCUSSION

Germination parameters

Table 4 shows the results obtained in the germination variables. The variables median germination time, germination percentage, coefficient of velocity of germination, and germination index were not different between the evaluated treatments.

In the variable daily total germination (DTG), differences were recorded between treatments, observing that the ConvAg treatment exceeded the ColdAg by 128.9% (Table 4). The optimal temperatures for germination of cabbage seeds range between 15 and 25 °C; these temperatures coincide with those of the ConvAg treatment (20-25 °C). With temperatures equal to or greater than 30 °C, germination can be accelerated; however, these conditions are detrimental to the initial development of the seedling (Červenski *et al.*, 2022). In this study, it was observed that thermal stress due to soil cooling and heat from the environment (ColdAg) delays the germination process and therefore prolongs it, which can impact the total germination percentage. It is important to highlight that, although the germination percentage was not statistically different between treatments, it is 4% higher in ColdAg than in ConvAg. This may be due to the fact that soil cooling causes sufficient moisture in the substrate or soil for the germination process to take place without adverse effects (Červenski *et al.*, 2022).

Biomass weights

The treatments significantly affected the fresh and dry biomass production of the seedlings that were harvested 23 d after sowing (Figure 2).

Table 4. Seed germination indicators of cabbage (*Brassica oleracea* var. *capitata* L.) under cold-water agriculture (ColdAg) and conventional agriculture (ConvAg) conditions.

Treatment	DTG	T_{50} (days)	GP	CVG	GI
ColdAg	1.87±0.49 b	5	66±1.04 a	16.61±1.04 a	6.17±0.35 a
ConvAg	4.28±0.64 a	5	62±0.55 a	19.42±0.77 a	5.40±0.26 a

Means ± SE with a different letter in each row indicate that there are statistical differences. Letters in each row with different letters are statistically different (Tukey, $p \leq 0.05$), n=3. DTG: Daily total germination; T_{50}: Median germination time; GP: Germination percentage, CVG: Coefficient of velocity of germination; GI: Germination index.

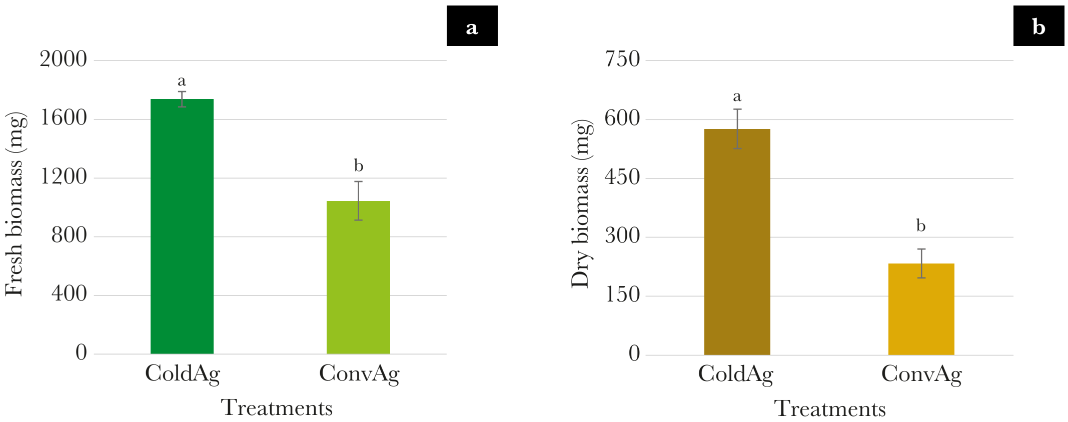

Figure 2. Weight of fresh (a) and dry (b) biomass of cabbage (*Brassica oleracea* var. *capitata* L.) seedlings per experimental unit, under cold-water agriculture (ColdAg) and conventional agriculture (ConvAg) conditions. Means ± SE with different letters in each subfigure indicate that there are statistical differences (Tukey, $p \leq 0.05$), n=3.

The fresh seedling biomass in the ColdAg treatment exceeded the ConvAg treatment by approximately 66.3% (Figure 2a), while the dry seedling biomass of the ColdAg treatment was 149.7% higher than that of ConvAg (Figure 2b). Soil cooling generates condensation of water vapor existing between the soil pores and allows sufficient humidity for the roots to absorb and transport water, generating greater seedling development (Craven and Weaver, 1998; Sabri *et al*., 2018; Sha'Arani *et al*., 2021).

When high ambient temperatures are present (*i.e*., 35-37 °C), the germination percentage for cabbage seeds decreases by more than 50% (Elson *et al*., 1992). However, if high ambient and low soil temperatures are combined, better conditions for germination can be achieved, since relative humidity can increase by 20% and thus sufficient soil moisture can be achieved (Červenski *et al*., 2022).

In the present experiment, the soil was cooled to 14.8 °C and the relative humidity was 85% inside the growth chamber, which increased the production of fresh and dry biomass of seedlings. Cool soil temperature allows for better growth and development of cabbage seedlings, especially at temperatures between 13 and 21 °C (Červenski *et al*., 2022). It has been demonstrated that periods with a warm shoot but cold root system, especially at high elevations in the morning hours, may allow relatively high photosynthetic activity despite cold soil (Göbel *et al*., 2019). Nevertheless, the physiological mechanisms which allow plant species to maintain a relatively high root water uptake at a low soil temperature are not yet fully understood and deserve further investigation.

CONCLUSIONS

Although the cold-water agriculture system (ColdAg) can prolong the germination period of cabbage seeds, it presents better results in the production of fresh and dry biomass of seedlings compared to seedlings grown under conventional agriculture (ConvAg) conditions.

In future studies it will be necessary to research the physiological, biochemical, and molecular processes that ColdAg triggers in the plant, and that can explain in greater detail the productive responses observed.

ColdAg technology represents a potential alternative to guarantee food security for a growing global population, in production conditions increasingly affected by global climate change.

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