New techniques to induce flowering and produce seeds of foreign cabbage varieties as a main step for a superior hybrids production

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ABSTRACT

This study aimed to reach a successful protocol for pushing the hard-flowered cabbage variety (Brunswick) under conditions of unavailability of cold needs and the number of hours of lighting in Egypt, it can be introduced into breeding programs to obtain Egyptian varieties and hybrids compatible with the Egyptian climatic conditions. This protocol allows seed production regardless of location or season. This study was carried out at Kaha Vegetable Research Farm (Kaluobia Governorate, Egypt). Two genotypes, Balady cv. (a local cabbage cultivar which highly susceptible to bolting) and Brunswick cv. (a foreign cabbage cultivar – which highly resistant to bolting) was used. This study was conducted in three separate experiments as follows: Experiment 1. Induction and generation of flowering in cabbage plants by seed vernalization and gibberellic acid treatment. Experiment 2. Induction and generation of flowering in cabbage plants by grafting and gibberellic acid treatment. Experiment 3. Induction and generation of flowering in cabbage plants by drought shock loading and gibberellic acid treatment. Averages of traits related to bolting, flowering and seed yield had been recorded. It could be concluded that the observed responses to flowering inducement treatments for pushing the hard-flowered cabbage varieties (Brunswick) - under conditions of unavailability of cold needs and the number of hours of lighting in Egypt – recorded good results for traits that measure flowering state and seed production. The best number (This is in keeping with this study) of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of Seed yield per plant, Weight of Seed yield per plant, Seed germination percentage (183, 103.41, 91.62, 244.56, 2761.33, 12.33 and 89.31) was found respectively in the treatment that received drought shocked loading with 300 ppm GA3 of Brunswick cabbage cultivar.

Based on the results obtained, three practical protocols were proposed that correspond to the Egyptian climatic conditions in terms of the length of the day and the temperature prevailing during the months of the year. These three protocols are presented in the form of schematic diagrams.

Keywords: Cabbage, Brassica oleracea var. capitata, bolting, flowering, seed setting, grafting, drought shock loading.

1. Introduction

Cabbage (Brassica oleracea var. capitata) originates from the south and western coast of Europe. Annual Egypt production is about 0.5 million tons of fresh heads from 15876 hectare; while, Annual world production is about 70 million tons of fresh heads from 2.5 million hectare (FAOSTAT, 2019).

There are many health benefits of cabbage such as cancer prevent, reduces risks of Alzheimer’s disease and help in weight loss (Roy, 2019).
High production of cabbage crop requires a cool, humid climate. The length of the total growing period varies between 90 (spring-sown) and 200 (autumn-sown) days, depending on climate, variety and planting date, but for good production the growing period is about 120 to 140 days. Most varieties can withstand a short period of frost of -6°C, some down to -10°C. Long periods (30 to 60 days) of -5°C are harmful. The plants with leaves smaller than 3 cm will survive long periods of low temperature but when the leaves are 5 to 7 cm, the plant will initiate a seed stalk and this leads to a poor-quality yield. Optimum growth occurs at a mean daily temperature of about 17°C with daily mean maximum of 24°C and minimum of 10°C. Mean relative humidity should be in the range of 60 to 90 percent (FAO, n.d.).

Because cabbage is one of the crops that are affected by vernalization (plants are induced to flower after exposure to low temperatures), these plants become sensitive to this phenomenon when it reaches a certain size with a number of true leaves, and also requires a long period low with temperature exposure for flowering. In Egypt, strong vernalization requirements lead to a delay in the breeding cycle due to the failure of foreign cabbage varieties to flowering.

Most biennial and winter annual vegetable crops are cool-season crops and have their origins in the Mediterranean region and grow best at temperatures between 50- and 70-degrees Fahrenheit. Many are also long-day plants, meaning that the transition to flowering or bulbing is stimulated only after day lengths exceed a critical day length in late spring or early summer. In addition to day length requirements, most winter-hardy biennial and perennial crops in our growing region need a certain number of hours at cool temperatures after they have grown to a certain size before they will flower. This is a process called vernalization. The time (or leaf stage) before a crop becomes responsive to vernalizing temperatures is called its juvenile period. Not all vegetable biennials have a juvenile period, and some can even vernalize as a seed. For some spring planted biennial crops, a string of cool days or nights can precondition them to flower sooner than desired. This unwanted, premature flowering is called bolting in most crops. Some crops will also reverse their progress towards flowering when higher temperatures interrupt the cold ones and full vernalization has not been reached, and this is called de-vernalizing. However, other environmental stresses can also push the plants to bolt, including disease, drought and nutrient deficiencies (Brainard, 2020). Furthermore, cabbage is a polycarpic plant, which has ability to turn back to vegetative growth after flowering (de-vernalization phenomenon); thus, reversion from reproductive growth to vegetative growth tends to occur when vernalization is insufficient (Ito and Saito, 1961). Research on floral induction by artificial low-temperature treatment (Wang et al., 2000) and gibberellins treatment (Nyarko et al., 2007) has been conducted with the intention of producing seed in tropical regions where cabbage seed production is difficult under natural conditions. It is possible to develop a method that enables to stimulate flowering in cabbage while disregarding the requirement of treating plants at low temperatures, thus we can remove regional restrictions on cabbage breeding and seed production.

Grafting is a method of flower induction under conditions that do not depend on low temperature conditions in several plant species (Chailakhyan, 1937; Zeevaart, 1976). Kagawa (1957) reported that he could induce cabbage flowering without low-temperature treatment by grafting it on a flowering radish (Raphanus sativus) rootstock; Who also added, when grafted onto flowering radish rootstocks, one in five cabbage scions flowered without low-temperature treatment; however, when grafted on flowering cabbage, no cabbage scions flowered. Kagawa (1957) discusses that this difference in floral induction of the grafted cabbages, may be caused by the difference in transmissibility of floral stimulating agents between radish and cabbage used as a rootstock. On the other hand, Hamamoto and Yoshida (2012) reported that cabbage did not flower at all when grafted onto flowering radish, these results suggest that it may be possible to induce flowering of cabbage without low-temperature treatment by grafting onto flowering plants, but success may vary depending on the plant species and/or cultivars used as a rootstock. It allows seed production regardless of location or season. In order to, identify rootstock plants suitable for our non-vernialization flowering method in cabbages, the flowering response of cabbages grafted onto radishes (Motoki et al., 2019).

Bolting is a common problem experienced by lettuce growers. It is caused by high temperatures, long periods of high light intensities, and drought. Lettuce has an internal counter that keeps track of the number of daylight hours the plant receives. Once a critical number of hours are received, the plant sends up its flower stalk. The exact number of hours varies from cultivar to cultivar. Apparently, lettuce goes through four distinct stages of growth; juvenile/vegetative, adult/vegetative, adult/intermediate, and adult/reproductive. The plant can handle environmental stresses quite well when vegetative.
However, once the intermediate stage is reached, environmental stresses, such as high temperatures or drought, will cause the plant to bolt (Rindels et al., 1994).

The final objective of this study is to adjust traditional vernalization method for cabbages heads (an expensive method) by using the methods of seed vernalization, gibberellic acid, grafting, and drought shock loading which enables to obtain strains of foreign cabbage. These strains can be involved in breeding programs to obtain Egyptian varieties and hybrids compatible with the Egyptian climatic conditions.

2. Materials and Methods

This study was carried out at Kaha Vegetable Research Farm (Kalubia Governorate, Egypt) and growth chambers for the technology of Vegetable Seed Production Department, Dokki, Giza Governorate, Egypt during four years field trial (winter of 2014/2015 to winter of 2017/2018). The experiment has gone through several stages, and some transactions have been modified to reach its main goal. The soil type of the experimental site is classified as a clay soil. Two genotypes, Balady cv. (a local cabbage cultivar - a cultivar highly susceptible to bolting) and Brunswick cv. (a foreign cabbage cultivar – a cultivar highly resistant to bolting) was used. Seeds were obtained from the Vegetable Seeds Production Unit, Vegetable Research Departments, Dokki, Giza, Egypt. Maximum, minimum and average high and low temperatures, relative humidity, daylight and rainfall in Table (1) were obtained from weather-atlas.com.

Table 1: Average temperatures, relative humidity, daylight and rainfall of field trials in Kaha Vegetable Research Farm, Kaha, `Kalubia Governorate, Egypt for evaluation of the high temperature in early season response in cabbage genotypes.

| Months     | Temperature (°C) | Relative humidity (%) | Daylight (hours) | Rainfall (mm) |
|------------|------------------|----------------------|------------------|--------------|
|            | Maximum  | Minimum  |                  |               |             |
| January    | 19.2°C  | 6.7°Cb  | 81                | 10.5h        | 5mm         |
| February   | 20.9°C  | 7.2°Cb  | 78                | 11.1h        | 4mm         |
| March      | 23.7°C  | 9.5°C   | 71                | 12h          | 3mm         |
| April      | 27.9°C  | 12°C    | 67                | 12.9h        | 1mm         |
| May        | 31.9°C  | 15.6°C  | 64                | 13.7h        | 1mm         |
| June       | 34.3°C  | 18.6°C  | 70                | 14.1h        | 0mm         |
| July       | 34.6°C  | 20.4°C  | 77                | 13.9h        | 0mm         |
| August     | 34.4°C  | 20.4°Cb | 79                | 13.2h        | 0mm         |
| September  | 32.3°C  | 18.4°Cb | 78                | 12.3h        | 0mm         |
| October    | 29.9°C  | 16.1°Cb | 79                | 11.4h        | 1mm         |
| November   | 25.4°C  | 13.2°Ca | 72                | 10.6h        | 2mm         |
| December   | 20.8°C  | 8.5°Ca  | 81                | 10.2h        | 6mm         |

°C = common season. °C = Date of cultivation of tolerant varieties for bolting under high temperature conditions in Egypt

This study was conducted in three separate experiments as follows:

2.1. Experiment 1. Induction and generation of flowering in cabbage plants by seed vernalization and gibberellic acid treatment.

Seeds of each of two varieties of cabbage (Brassica oleracea var. capitata L.) were imbibed in a solution containing 302.44 g/l polyethylene glycol 6000 (PEG 6000). For each variety, 20 seeds were placed in each of three replicates 5 cm-Petri dishes lined with three filter papers, which were then moistened with 20 ml PEG 6000 solution. The experiment began in mid-October. The seeds were exposed to vernalization temperatures (2°– 5°C) and 8-h light (40 μmol m⁻² s⁻¹) Where it means, μmol (micromoles) m⁻² (per square meter) s⁻¹ (per second) or 1739 lux) for 8 weeks using a growth chamber. Control seeds were primed in 302.44 g/l PEG 6000 for 11 day immediately prior to planting, to ensure that the vernalized and control seeds had been primed for the same thermal time (°C d). At planting, the seeds were washed in sterile water and sown immediately in 4 cm-square plastic modular trays filled with compost. After 45 day, seedlings were transplanted into the field. Plants were arranged in the plots with three replicates. GA₃ applications started 45 days after transplanting, and seven
applications were made at one-week intervals. In each block, a plant from the vernalized-seed and the control treatment of each variety was sprayed until run-off with a GA$_3$ solution, and another plant of each treatment with distilled water, using a hand sprayer. Two drops of Tween 80 (wetting agent) were added to each liter. The GA$_3$ solutions were prepared at 300 mg/l by dissolving 300 mg of GA$_3$ in 10 ml ethanol and making this up to 1 liter with distilled water. A two concentration of gibberellic acid has been used (0 and 300 ppm) (Nyarko et al., 2007).

2.2. Experiment 2. Induction and generation of flowering in cabbage plants by grafting and gibberellic acid treatment.

Egyptian commercial cabbage cultivar, “Balady cabbage” (Brassica oleracea var. Capitata) and Egyptian commercial Radish cultivar, “Balady radish” (Raphanus sativus L. var. longipinnatus) were used as rootstock plants for grafting experiment. These cultivars were chosen because flowering induction is easy under Egypt conditions and it do not need low-temperature treatment for flowering even at the seed stage. Foreign commercial cabbage cultivar “Brunswick” was used as scion plants for grafting experiment. To reduce the effect of juvenility of the scion on its flowering response, the seedlings grown for more than 10 weeks after sowing were used for collecting scions. The terminal shoots of the seedlings of scions were pinched to elongate the lateral shoots, which were used as scions. All plants were irrigated and fertilized. The stem of the rootstock (Balady cabbage rootstocks and Balady radish rootstocks) was cut at a height of 3–5 cm from the top of the hypocotyls, and the cabbage scion with 2–3 expanded leaves was grafted onto the stem of the rootstock by cleft grafting. After grafting, the scion and a portion of the leaves of the rootstock plant were covered with a clear polyethylene bag to maintain high humidity, and the plants were grown under nursery and field conditions; respectively. One to two weeks after grafting, once the scion and rootstock were fully connected, the polyethylene bag was removed. To promote translocation of assimilates from the rootstock to the scion, all the lateral shoots of the rootstock were removed and new leaves on the scion were removed with only 3–4 newly expanded leaves remaining, so that scion can keep sink activity. GA$_3$ applications started 45 days after transplanting of Brunswick cabbage cultivar (scion), and seven applications were made at one-week intervals. A two concentrations of gibberellic acid has been used (0 and 300 ppm). Spraying with gibberellin is done on scion plants only (Motoki et al., 2019).

2.3. Experiment 3. Induction and generation of flowering in cabbage plants by drought shock loading and gibberellic acid treatment.

This experiment is summarized in several steps; as follow, the seeds of the Brunswick and Balady cabbage cultivar (as control) were sown at the nursery in mid-May. The seedlings stayed in the nursery for 45 days. Seedlings were transferred to the field (on the 1st of July). All plants were irrigated and fertilized for two full months (July and August); then, Irrigation was prevented completely for two full months (September and October). GA$_3$ applications started 45 days after transplanting, and seven applications were made at 1-week intervals (on the mid-August). Two concentrations of gibberellic acid have been used (0 and 300 ppm). All grafted Brunswick on both of rootstocks (cabbage-radish) and un grafted were sprayed with (0 and 300 ppm) gibberellin at 45 days from transplanting. After the end of the period to prevent irrigation from the plants, irrigation and fertilization for plants were done at regular intervals to the end of plant life.

It is worth mentioning that these protocols have been adapted to suit the day length (photoperiod) and the temperature under unfavorable conditions of cold needs and the number of hours of lighting in Egypt by making use of many previous studies that discussed the issue of flowering cabbage under unfavorable conditions.

Data were recorded on several quantitative traits consisting of Number of days from planting to bolting with visible flower buds (days), Flower stalk height (cm), Percentage of flowering plants (%), Days to beginning of seed harvesting (days), No. Seed yield per plant, Weight of Seed yield per plant (g), Seed germination (%), Number of successful grafting (plant/plant), Number of days from grafting to bolting with visible flower buds (days).
Table 2: Mean comparisons of morphological traits in two cabbage cultivars under Egypt conditions.

| Traits                                          | Balady cv. | Brunswick cv. |
|------------------------------------------------|------------|---------------|
| Fresh weight of the entire plant (kg)           | 8.500      | 3.800         |
| Weight of non-edible plants (wrapper leaves + stem) (kg) | 3.300      | 1.700         |
| Net head weight (kg)                            | 5.500      | 2.100         |
| Stem height from wrapper leaves to head (cm)     | 31         | 18            |
| Stem height up to wrapper leaves (cm)            | 21         | 11            |
| Wrapper leaf No./ plant                         | 27         | 16            |
| Wrapper leaf wt./ plant (kg)                     | 2.900      | 1.800         |
| Head height (cm)                                | 30         | 20            |
| Head diameter (cm)                              | 75         | 45            |
| Day from transplanting dates to 50% harvest      | 160        | 90            |
| Day from transplanting dates to the end of harvest | 210        | 120           |

2.4. Experimental design and Statistical analysis:

The data collected were analyzed statistically using Fisher's analysis of variance technique (described a procedure for pairwise comparisons called the least significant difference (LSD) test). This test is to be used only if the hypothesis that all means are equal is rejected by the overall F test. If the overall test is significant, a procedure analogous to ordinary Student's t test is used to test any pair of means. If the overall F ratio is not significant, no further tests are performed. When it is used, the two treatments will be declared different if the absolute difference between two sample means by using combined ANOVA across years with one way randomized blocks analysis (Multiple comparisons and trends among treatment means, n.d.) Two tests were used to compare the difference among the treatment means; namely, Duncan (used a different approach to compare means, called the multiple range test and Unpaired two-tailed Student’s t-test (is used to test any pair of means), at 5% level of probability was employed to compare means) (Gomez and Gomez, 1984).

The two-factor experiment (seed vernalization and GA$_3$, grafting and GA$_3$ and drought shock loading and GA$_3$) consisting of treatment combinations was laid out in Randomized Complete Block Design (RCBD) with three replicates. The treatment combinations were randomly placed to unit plots in each block. The plot area was 16.2 m$^2$, contained 6 ridges. Each ridge was 90 cm wide, 3.0 m long and 50 cm for plant spacing, leaving a guard raw between the experimental units. Treatments in experiment were randomly arranged in a complete blocks design with three replicates. Each replicate consisted of thirty-six plants.

2.5. Data Collection:

Data were collected from selected plants in each unit plot. To avoid border effect with the highest precision six plants were selected randomly from each plot out of 30 discarding the outer two rows and outer plants of the middle lines.

2.5.1. Height of flowering stalk:

It was measured from ground level to the tip of the flowering stalk before harvest, and the average was recorded in centimeter (cm) from selected plants.

2.5.2. Percent flowering plants:

The number of plants flowered was conducted at 5 days interval from initiation to completion. Percentage was determined by the following formula:

\[
\text{Percent flowering plants} = \frac{\text{No. of plants flowered}}{\text{Total no. of plants in the plot}} \times 100
\]

2.5.3. Weight of seeds per plant:

The seed were collected, weighed and recorded from randomly selected six plants and the mean weight was Yield of seeds per plant expressed in gram (g).
2.5.4. Germination percentage:
The number of seeds germinated was recorded respectively for each petri dish daily. Seeds were considered to have germinated when radical emerged about 2 mm from the seed. The germination percentage was determined by the following formula:

\[
\text{Germination percentage} = \left( \frac{\text{No. of seeds germinated}}{\text{No. of seeds set for germination}} \right) \times 100
\]

3. Results and Discussion

3.1. Combined Effects of seed vernalization and gibberellic acid on bolting, flowering and seed setting of Brunswick and Balady cabbage cultivars.

There was a significant difference in respect of number of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of Seed yield per plant, Weight of Seed yield per plant and Seed germination percentage at different levels of seed vernalization and GA3. The maximum number of days from planting to bolting with visible flower buds, Days to beginning of seed harvesting (131, 183.21) was found respectively in the treatment that received seed vernalization with 300 ppm GA3 of Brunswick cabbage cultivar (Table 3).

Table 3: Combined effects of seed vernalization and gibberellic acid on bolting, flowering and seed setting of Brunswick and Balady cabbage cultivars.

| Cultivar  | Treatment combination (seed vernalized + GA3) | Number of days from planting to bolting with visible flower buds (days) | Height of flowering stalk (cm) | Percentage of flowering plants (%) | Days to beginning of seed harvesting (days) |
|-----------|-----------------------------------------------|-----------------------------------------------------------------------|-------------------------------|-----------------------------------|------------------------------------------|
| Balady cv. | Non - seed vernalized + 0 ppm GA3             | 79.3b                                                                | 160.70a                      | 100%a                             | 154.36b                                  |
| Brunswick cv. | Non - seed vernalized + 0 ppm GA3          | 0                                                                    | 0                             | 0%                                | 0                                        |
|          | Non - seed vernalized + 300 ppm GA3        | Bolting only                                                          | 0                             | 0%                                | 0                                        |
|          | Seed vernalized + 0 ppm GA3               | 0                                                                    | 0                             | 0%                                | 0                                        |
|          | Seed vernalized + 300 ppm GA3             | 131a                                                                 | 96.66b                       | 83.56%b                           | 183.21a                                  |

LSD 0.05: 1.194 3.022 1.999 2.926

Means within columns followed by the same letter are not statistically different at 5% level (Unpaired two-tailed Student’s t-test.).

Table 3: Continued

| Cultivar  | Treatment combination (seed vernalized + GA3) | Number of Seed yield per plant | Weight of Seed yield per plant (g) | Seed germination (%) |
|-----------|-----------------------------------------------|-------------------------------|-----------------------------------|----------------------|
| Balady cv. | Non - seed vernalized + 0 ppm GA3             | 4448.31a                     | 20.45a                           | 94.63a               |
| Brunswick cv. | Non - seed vernalized + 0 ppm GA3         | 0                             | 0                                | 0                    |
|          | Non - seed vernalized + 300 ppm GA3         | 0                             | 0                                | 0                    |
|          | Seed vernalized + 0 ppm GA3                 | 0                             | 0                                | 0                    |
|          | Seed vernalized + 300 ppm GA3               | 2527.11b                     | 15.7b                           | 88.75b               |

LSD 0.05: 130.105 1.665 2.389

Means within columns followed by the same letter are not statistically different at 5% level (Unpaired two-tailed Student’s t-test.).
The maximum number of heights of flowering stalk, Percentage of flowering plants, number of Seed yield per plant, Weight of Seed yield per plant and Seed germination percentage (160.70, 100, 4448.31, 20.45 and 94.63) was observed respectively in the treatment that received no seed vernalization and no GA$_3$ of Brunswick cabbage cultivar. While, failure to respond to induction of flowering recorded in the treatments that received no seed vernalization with no GA$_3$, no seed vernalization with 300 ppm GA$_3$ (bolting only) and seed vernalization with no GA$_3$ of Brunswick cabbage cultivar.

The best number (This is in keeping with this study) of number of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of Seeds yield per plant, Weight of Seeds yield per plant and Seed's germination percentage (131, 96.66, 83.56, 183.21, 2527.11, 15.7 and 88.75) was found respectively in the treatment that received seed vernalization with 300 ppm GA$_3$ of Brunswick cabbage cultivar. Research on floral induction by artificial low-temperature treatment (Wang et al., 2000) and gibberellins treatment (Nyarko et al., 2007) has been conducted with the intention of producing seed in tropical regions where cabbage seed production is difficult under natural conditions. Among the many Brassica species, there is a wide range of ‘vernalization requirements’ for flowering and additionally the seedling had to pass through a juvenile phase during which it cannot be induce flowering (Cheng and More 1967; Friend 1985; Chandler and Dean 1994). Seed vernalization alone did not induce flowering, and had no effect on stem height. GA$_3$ alone increased the stem height of almost all cabbage varieties, but did not induce flowering (Nyarko et al., 2007). Ito et al., 1966; Friend, 1985; Lin et al., 2005) suggest that cold treatment alone will not cause vernalization of cabbage seeds. Examining the cold treatment of seeds, combined with other flower-inducing factors, may be more effective. Gibberellic acid (GA$_3$) promotes flowering in many plants (Hanks, 1985; Halevy, 1990; Kurtar and Ayan, 2005). Combining GA$_3$ with cold treated seeds may prove beneficial for flowering in cabbage, and allow seed production in the tropics (Nyarko et al., 2007).

3.2. Combined Effects of grafting and gibberellic acid on bolting, flowering and seed setting of Brunswick and Balady cabbage cultivars.

There was a significant difference in respect of number of successful grafting (plant/plant), percentage of flowering plants, number of days from grafting to bolting with visible flower buds, days to beginning of seed harvesting, no. Seed yield per plant, weight of Seed yield per plant and Seed germination percentage. at different levels of grafting and GA$_3$ (Table 4).
### Table 4: Combined effect of grafting and gibberellic acid on bolting, flowering and seed setting of Brunswick and Balady cabbage cultivars.

| Cultivar          | Treatment combination (Grafting + Rootstock + GA3) | Number of successful grafting (plant/plant) | Flowering plants (%) | Number of days from grafting to bolting with visible flower buds (days) | Days to beginning of seed harvesting (days) |
|-------------------|---------------------------------------------------|--------------------------------------------|----------------------|---------------------------------------------------------------|-------------------------------------------|
| Balady cv.        | Non-grafted plants + 0 ppm GA3                     | 0                                          | 100b                 | 0                                                             | 142.75a                                   |
|                   | Non-grafted plants + 0 ppm GA3                     | 0                                          | 0                    | 0                                                             | 0                                         |
|                   | Non-grafted plants + 300 ppm GA3                   | 0                                          | Bolting only         | 0                                                             | 0                                         |
| Brunswick cv.     | Balady cabbage cv. (rootstock) + 0 ppm GA3         | 57/60                                      | 5.50e                | 40.52c                                                        | 87.88d                                    |
| (scion)           | Balady cabbage cv. (rootstock) + 300 ppm GA3       | 58/60                                      | 9.96d                | 40.72c                                                        | 88.61d                                    |
|                   | Balady Radish cv. (rootstock) + 0 ppm GA3          | 56/60                                      | 28.913c              | 43.22a                                                        | 94.59b                                    |
|                   | Balady Radish cv. (rootstock) + 300 ppm GA3        | 55/60                                      | 79.057b              | 42.25b                                                        | 92.00c                                    |
| LSD 0.05          | -                                                 | 0.303                                     | 0.972                | 1.033                                                         |                                           |

Mean followed by the same letter(s) are not significantly different at the 5% level of probability (Duncan’s multiple range test).

### Table 4: Continued

| Cultivar          | Treatment combination (Grafting + Rootstock + GA3) | No. Seed yield per plant | Weight of Seed yield per plant (g) | Seed germination (%) |
|-------------------|---------------------------------------------------|--------------------------|-----------------------------------|---------------------|
| Balady cv.        | Non-grafted plants + 0 ppm GA3                     | 3784.30b                 | 17.71b                            | 95.78a              |
|                   | Non-grafted plants + 0 ppm GA3                     | 0                        | 0                                 | 0                   |
|                   | Non-grafted plants + 300 ppm GA3                   | 0                        | 0                                 | 0                   |
|                   | Balady cabbage cv. (rootstock) + 0 ppm GA3         | 119.56d                  | 0.585e                            | 86.49d              |
| Brunswick cv.     | Balady cabbage cv. (rootstock) + 300 ppm GA3       | 242.24d                  | 2.447d                            | 88.70e              |
| (scion)           | Balady Radish cv. (rootstock) + 0 ppm GA3          | 854.45c                  | 3.62c                             | 90.36b              |
|                   | Balady Radish cv. (rootstock) + 300 ppm GA3        | 1819.43b                 | 10.71b                            | 90.60b              |
| LSD 0.05          | 125.983                                           | 0.355                    | 0.662                             |                     |

Mean followed by the same letter(s) are not significantly different at the 5% level of probability (Duncan’s multiple range test).

The maximum Number of successful grafting (plant/plant) (58/60) was observed in the treatment that received grafted plants (Brunswick cv. as scion/Balady cabbage cv. as rootstock) with 300 ppm GA3. The maximum number of percentages of flowering plants, days to beginning of seed harvesting, no. Seed yield per plant, weight of Seed yield per plant and seed germination percentage (100, 142.75, 3784.30, 17.71 and 95.78) was found respectively in the treatment that received non-grafted plant with no GA3 of Balady cabbage cultivar. While, failure to respond to induction of
flowering recorded in the treatments that received non-grafted plant with no GA$_3$ and 300 ppm GA$_3$ (bolting only) of Brunswick cabbage.

The best number (This is in keeping with this study) of successful grafting (plant/plant), percentage of flowering plants, number of days from grafting to bolting with visible flower buds, days to beginning of seed harvesting, no. Seed yield per plant, weight of Seed yield per plant and Seed germination percentage (55/60, 79.057, 42.25, 92.00, 1819.43, 10.71 and 90.60) was found respectively in the treatment that received grafted plants (Brunswick cv. as scion/Balady radish cv. as rootstock) with 300 ppm GA$_3$ of Brunswick cabbage cultivar. Grafting is a known method for floral induction under non-inductive conditions in several kinds of plants (Chailakhyan, 1937; Zeevaart, 1976). Kagawa (1957) reported that he could induce cabbage flowering without low-temperature treatment by grafting it on a flowering radish (Raphanus sativus) rootstock. On the other hand, a different publication reported that cabbage did not flower at all when they were grafted onto flowering radish (Hamamoto and Yoshida, 2012). It's suggested that it may be possible to induce flowering of cabbage without low-temperature treatment by grafting onto flowering plants, but success may vary depending on the plant species and/or cultivars used as a rootstock (Motoki et al., 2019).

These results indicate that it may be possible to induce flowering of cabbage without low-temperature treatment by grafting on flowering plants in addition to, using gibberellic acid to compensate for the long light day.

When the cambium of the scion joins fully with that of the rootstock, intact cells divide and proliferate into calli, which eventually differentiate into vasculature and plasmodesmata forms (Melnyk and Meyerowitz, 2015). Although the detailed molecular mechanisms underlying this process require further research, some studies have found that hormones, such as auxin, cytokinin and GA$_3$, play a pivotal role in regulating stock–scion interactions (Aloni et al., 2010). Histological and microarray analyses of Arabidopsis micrografting identified auxin, ethylene and jasmonic acid as important molecules that participate in development of the graft union, and a model has been proposed to better interpret this phenomenon (Yin et al., 2012).

Grafting experiments using two varieties of (Pharbitis nil and Perilla frutescens var. crispa) revealed that a transmissible flowering stimulus is involved in stress-induced flowering (Wada and Takeno, 2010).

3.3. Combined effect of drought shock loading and gibberellic acid on bolting, flowering and seed setting of Brunswick and Balady cabbage cultivars.

There was a significant difference in respect of number of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of seed yield per plant, Weight of seed yield per plant and Seed germination percentage at different levels of drought shock loading and gibberellic acid (Table 5).

The maximum number of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of seed yield per plant, Weight of seed yield per plant and Seed germination percentage (191.54, 154.22, 100, 246.32, 4195.51, 19.14 and 94.17) was found respectively in the treatment that received non-drought shocked loading with zero GA$_3$ of Balady cabbage cultivar. While, failure to respond to induction of flowering recorded in the treatments that received non-drought shocked loading with zero ppm GA$_3$, non-drought shocked loading with 300 ppm GA$_3$ (bolting only) and drought shocked loading with zero GA$_3$ (bolting only) of Brunswick cabbage cultivar.

The best number (This is in keeping with this study) of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of seed yield per plant, Weight of seed yield per plant and Seed germination percentage (186.74, 102.31, 93, 244.14, 2752.64, 12.43 and 88.35) was found respectively in the treatment that received drought shocked loading with 300 ppm GA$_3$ of Brunswick cabbage cultivar.

Due to water deficits, the physiology of crop is disturbed which causes a large number of changes in the morphology and anatomy of plants. Drought stress is a major limiting factor for plant growth and development worldwide (Belhassen, 1995). Many plant species can be induced to flower by responding to stress factors. Flowering in many plant species is regulated by environmental factors, such as night-length in photoperiodic flowering and temperature in vernalization. On the other hand, a short-day (SD) plant such as Pharbitis nil (synonym Ipomoea nil) can be induced to flower under long...
days (LD) when grown under poor-nutrition, low-temperature or high-intensity light conditions. The flowering induced by these conditions is accompanied by an increase in phenylalanine ammonia-lyase (PAL) activity (Wada and Takeno, 2010). The flowering induced by these conditions is accompanied by an increase in phenylalanine ammonia-lyase (PAL) activity (Hirai et al., 1995).

Taken together, these facts suggest that the flowering induced by these conditions might be regulated by a common mechanism. Poor nutrition, low temperature and high-intensity light can be regarded as stress factors, and PAL (Phenylalanine ammonia-lyase inhibitors) PAL activity increases under these stress conditions (Dixon and Paiva, 1995).

**Fig. 2:** Combined effect of grafting (Brunswick cv. as scion/Balady radish cv. as rootstock) and gibberellic acid (C) on bolting (D), flowering (E) and seed setting (F) of Brunswick cabbage cultivar.
Table 5: Combined effect of drought shock loading and gibberellic acid on bolting, flowering and seed setting of Brunswick and Balady cabbage cultivars.

| Cultivar         | Treatment combination (Drought shocked loading + GA₃) | Number of days from planting to bolting with visible flower buds (days) | Height of flowering stalk (cm) | Flowering plants (%) | Days to beginning of seed harvesting (days) |
|------------------|--------------------------------------------------------|------------------------------------------------------------------------|-------------------------------|----------------------|------------------------------------------|
| Balady cv.       | Non-drought shocked loading + 0 ppm GA₃                | 191.54<sup>a</sup>                                                   | 154.22<sup>a</sup>            | 100%<sup>a</sup>       | 246.32<sup>a</sup>                        |
| Brunswick cv.   | Non-drought shocked loading + 0 ppm GA₃               | 0                                                                     | 0                             | 0                    | 0                                        |
|                  | Non-drought shocked loading + 300 ppm GA₃             | Bolting only                                                          | 0                             | 0                    | 0                                        |
|                  | Drought shocked loading + 0 ppm GA₃                   | Bolting only                                                          | 0                             | 0                    | 0                                        |
|                  | Drought shocked loading + 300 ppm GA₃                 | 186.74<sup>b</sup>                                                   | 102.31<sup>b</sup>            | 93%<sup>b</sup>       | 244.14<sup>a</sup>                        |
| LSD 0.05         |                                                        | 3.421                                                                 | 1.772                         | 2.617                | 4.104                                    |

Means within columns followed by the same letter are not statistically different at 5% level (Unpaired two-tailed Student’s t-test).

Table 5: Continued

| Cultivar         | Treatment combination (Drought shocked loading + GA₃) | No. Seed yield per plant | Weight of Seed yield per plant (g) | Seed germination (%) |
|------------------|--------------------------------------------------------|--------------------------|-----------------------------------|----------------------|
| Balady cv.       | Non-drought shocked loading 0 ppm GA₃                  | 4195.51<sup>a</sup>      | 19.14<sup>a</sup>                 | 94.17<sup>a</sup>    |
| Brunswick cv.   | Non-drought shocked loading 0 ppm GA₃                  | 0                        | 0                                 | 0                    |
|                  | Non-drought shocked loading + 300 ppm GA₃             | 0                        | 0                                 | 0                    |
|                  | Drought shocked loading + 0 ppm GA₃                   | 0                        | 0                                 | 0                    |
|                  | Drought shocked loading + 300 ppm GA₃                 | 2752.64<sup>b</sup>      | 12.43<sup>b</sup>                | 88.35<sup>b</sup>    |
| LSD 0.05         |                                                        | 45.420                   | 0.634                             | 2.448                |

Means within columns followed by the same letter are not statistically different at 5% level (Unpaired two-tailed Student’s t-test).

Fig. 3: Combined effect of drought shock loading and gibberellic acid on bolting (G), flowering (H) and seed setting (I) of Brunswick cabbage cultivar.

3.4. A comparison of the combined effect of three different protocols on bolting, flowering and seed setting of Brunswick and Balady cabbage cultivar.

There was a significant difference in respect of number of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed...
harvesting, number of Seed yield per plant, Weight of Seed yield per plant and Seed germination percentage at the three best treatments on flowering and seed setting of Brunswick and Balady cabbage cultivars (Table 6).

The maximum number of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of Seed yield per plant, Weight of Seed yield per plant and Seed germination percentage (187.33, 157.35, 100, 247.33, 4276, 21.31 and 93) was observed respectively in the treatment that received non-treatment of Balady cabbage cultivar.

**Table 6: Comparison of the effect of the three best treatments on flowering and seed setting of Brunswick and Balady cabbage cultivar.**

| Treatments of protocols | Number of days from planting to bolting with visible flower buds (days) | Height of flowering stalk (cm) | Percentage of flowering plants (%) | Days to beginning of seed harvesting (days) |
|-------------------------|-------------------------------------------------|-------------------------------|-----------------------------------|------------------------------------------|
| Control (Balady cv.)    | 187.33<sup>a</sup>                              | 157.35<sup>a</sup>            | 100<sup>a</sup>                   | 247.33<sup>a</sup>                      |
| Seed vernalized + 300 ppm GA<sub>3</sub> (Brunswick cv.) | 129.66<sup>c</sup>                              | 94.61<sup>c</sup>             | 84<sup>c</sup>                     | 172<sup>b</sup>                         |
| (Balady radish cv. as Rootstock / Brunswick cabbage cv. as Scion) | 44<sup>d</sup>                                 | 39.59<sup>d</sup>             | 71.55<sup>d</sup>                 | 90.63<sup>c</sup>                      |
| Drought shocked loading + 300 ppm GA<sub>3</sub> (Brunswick cv.) | 183<sup>b</sup>                               | 103.41<sup>b</sup>            | 91.62<sup>b</sup>                 | 244.56<sup>a</sup>                     |
| LSD 0.05                | 2.898                                           | 2.926                         | 1.754                             | 15.245                                  |

Mean followed by the same letter(s) are not significantly different at the 5% level of probability (Duncan’s multiple range test).

The best number (This is in keeping with this study) of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of Seed yield per plant, Weight of Seed yield per plant and Seed germination percentage (183, 103.41, 91.62, 244.56, 2761.33, 12.33 and 89.31) was found respectively in the treatment that received drought shocked loading with 300 ppm GA<sub>3</sub> of Brunswick cabbage cultivar.

It could be concluded that the observed responses to flowering inducement treatments for pushing the hard-flowered cabbage varieties (Brunswick) - under conditions of unavailability of cold needs and the number of hours of lighting in Egypt – recorded good results for traits that measure flowering state and seed production. The best number (This is in keeping with this study) of days from planting to bolting with visible flower buds, height of flowering stalk, Percentage of flowering plants, Days to beginning of seed harvesting, number of Seed yield per plant, Weight of Seed yield per plant, Seed germination percentage (183, 103.41, 91.62, 244.56, 2761.33, 12.33 and 89.31) was found
respectively in the treatment that received drought shocked loading with 300 ppm GA$_3$ of Brunswick cabbage cultivar.

In addition to and Based on the results obtained, three practical protocols were proposed that correspond to the Egyptian climatic conditions in terms of the length of the day and the temperature prevailing during the months of the year. These three protocols are presented in the form of schematic diagrams; respectively, figure 4. Schematic diagram of the induction and generation of flowering in cabbage plants by seed vernalization and gibberellic acid treatment (protocol 1), figure 5. Schematic diagram of the Induction and generation of flowering in cabbage plants by grafting and gibberellic acid treatment (protocol 2) and figure 6. Schematic diagram of the Induction and generation of flowering in cabbage plants by drought shock loading and gibberellic acid treatment (protocol 3).

**Protocol (1):**

![Schematic diagram of the induction and generation of flowering in cabbage plants by seed vernalization and gibberellic acid treatment (protocol 1).](image)

**Fig. 4:** Schematic diagram of the induction and generation of flowering in cabbage plants by seed vernalization and gibberellic acid treatment (protocol 1).
**Protocol (2):**

Sowing of seeds
Brunswick cabbage cultivar (scion)
(15 September)

45 days Nursery

Seedling
To a larger pot
(Rotating)
(30 October)

(5 December)
Begin spraying
With gibberellic acid – seven
Applications
Were made at 1- week intervals

February
As scion with 2-3 expanded leaves was grafted on to the stem of the rootstock.

Grafting

Cut at a height of 3-5 cm from the top of the hypocotyls
(Rootstock)

Sowing of seeds
Balady radish cultivar (Rootstock)
(15 September)

45 days Nursery

Seedling
To a larger pot
(Rotating)
(30 October)

February

**Fig. 5:** Schematic diagram of the Induction and generation of flowering in cabbage plants by grafting and gibberellic acid treatment (protocol 2).
Fig. 6: Schematic diagram of the Induction and generation of flowering in cabbage plants by drought shock loading and gibberelic acid treatment (protocol 3).
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