Phenology of evening primrose (*Oenothera biennis* L.) as affected by vernalization and gibberellic acid (GA_3)

Bakhtyar Rezaee[1], Azim Ghasemnezhad[2]*, Ebrahim Zeinali[3]

1. Department of Horticulture and Forestry, Agriculture Faculty, Baghlan University, Baghlan Province, Afghanistan

2. Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Iran

3. Department of Agronomy, Gorgan University of Agricultural Sciences and Natural Resources, Iran

Abstract

Evening primrose is one of the herbal biennial medicinal and oilseed plants. Its seed oil is one of the important sources of omega-6 as well as gamma-linolenic acid. Encouraging the plant to flower without winter conditions is important in biennial plants. This experiment aimed to investigate the phenology of evening primrose as affected by vernalization and gibberellic acid. At the beginning of the experiment, seed samples were kept in refrigerator conditions (2 - 4 °C) for different periods of 0, 10, 20, 30, 40, 50, and 60 days. To study the influence of GA3 at the end of day 60, half of the vernalized seeds were treated with 500 ppm GA3 solution for 24 hours before they were planted. The time of seedling emergence, 4-, 6-, 8, and 10- leaf stages of seedling, stem formation, flowering, capsule formation, capsule filling, side stem production, physiological ripening of the seeds, capsule browning, and harvest were recorded. Results showed that while control plants did not flower, vernalization significantly influenced all of the phenological stages of the experimental plants. A combination of vernalization and GA3 significantly affected stem formation, side stems production, and capsule filling times. Finally, according to the results, seed priming with 2 to 4 °C moist-chilling for at least 10 days and GA3 in a minimum concentration of 500 ppm guarantee flower formation of seeds of evening primrose with late sowing time in spring.

Keywords: geographic data, gamma linoleic acid, maturity, rosette, seed oil, seed filling

Rezaee, B., A. Ghasemnezhad, E. Zeinali. 2023. 'Phenology of evening primrose (*Oenothera biennis* L.) as affected by vernalization and gibberellic acid (GA_3)*'. Iranian Journal of Plant Physiology 13* (3),4589- 4597.

Introduction

Evening primrose (*Oenothera biennis* L.) is a herbal biennial medicinal and oilseed plant. This plant flowers after sunset, which is why it is called evening primrose (Beites and Morgan, 2014). Evening primrose seed oil is one of the important sources of omega-6 as well as Gamma-Linolenic Acid (GLA) (Dietz et al., 2016). GLA is one of the most valuable fatty acids which is used in the pharmaceutical, cosmetic, and food industries (Ghasemnezhad and Honermeier, 2012; Greiner and Köhl, 2014; Sohrabi et al., 2017). Fatty acids, phenolic acids, flavonoids, protein, carbohydrates,

* *Corresponding Author*
E-mail Address: ghasemnezhad@gau.ac.ir
Received: April, 2023
Accepted: June, 2023

minerals, and vitamins are the main constituents of evening primrose (Timoszuk et al., 2018).

The plant has been cultivated in recent years in many countries around the world. As evening primrose is a biennial plant, it needs two seasons to produce seeds.

The cultivation of a plant in new regions requires the introduction of a new method. Treating seeds or seedlings at low temperatures to move from vegetative to a reproductive phase is a necessary process for the flowering of biennial and perennial plants in temperate regions (Woods et al., 2017). This phenomenon is called vernalization (Dixon et al., 2019). The duration of vernalization is important in plant flowering, for example, it has been reported that in turnip vernalization more than 40 days accelerates flowering. In contrast, seeds vernalized for less than 30 days did not flower (Zheng et al., 2018). The same was observed in beetroot (*Beta vulgaris*) (McCormick et al., 2014). Vernalization induced early flowering in *Dianthus barbatu* L. ((Dall'Agnese et al., 2014). Dhall (Dhall, 2017) reported that among 13 different genotypes of garlic (*Allium sativum* L.) which were incubated at 4 °C for two months, only nine genotypes were vernalized. At low-temperature conditions, onion plants produced flowers instead of bulbs (Fukuda et al., 2017). Light and temperature are two important factors in plant flowering. Many species of biennial vegetables require vernalization for flowering and seed production. Generally, the optimum vernalization temperature is between 3 °C to 10 °C, which varies from variety to variety and species to species. In red beet, it has been reported that the vernalization of seeds for 4-12 weeks, produced flowering plants compared to non-treated seeds (McCormick et al., 2014). This experiment aimed to investigate the phenology of evening primrose as affected by vernalization and gibberellic acid.

Material and Methods

The present work was conducted at Gorgan University of Agricultural Sciences and Natural Resources in a factorial base with a completely randomized design under the climate conditions of Gorgan with 3 replications. Seeds were obtained from the Research Center of the Gorgan University of Agricultural Sciences and Natural Resources. For each treatment around 10 g of seeds were selected and divided into two sterilized Petri dishes (each containing 5 g). The seeds were soaked in distilled water containing filter paper for 30 min and then the extra water was removed. Then, the Petri dishes were carefully wrapped with Parafilm. Afterwards, the samples were placed in 2 - 4 °C refrigerator conditions for cold treatment for 0, 10, 20, 30, 40, 50, and 60 days. To study the influence of gibberellic acid (GA_3), at the end of cold storage, half of the vernalized seeds were soaked in GA_3 (500 ppm) solution for 24 hours. Then, both the GA_3-treated and control (no GA_3) seeds were simultaneously cultivated in 6 kg pots containing 2:1:1 part of the soil, sand, and leaf compost in three replications. The EC and pH of the soil mixture were recorded at room temperature by an EC meter and a pH meter, respectively (Table 1). The phenological indexes including the times of seedling emergence, 4-, 6-, 8-, and 10-leaf stages of seedlings, stem formation, flowering formation, capsule formation, capsule filling, side stem, seeds' physiological maturity, ripening, and the harvesting were recorded. The capsule formation time was considered from the blossom stage to flower shattering.

Table 1
EC and pH values of used culture

Soil components	pH	EC	Room Temperature
Farm Soil	8.1	1.922 ms/cm	24 ℃
Sand	8.1	0.466 ms/cm	24 ℃
Leaf Compost	8.34	2.85 ms/cm	24 ℃
Soil Composition	8.33	4.39 ms/cm	24 ℃

Statistical analysis of the obtained data was done by SPSS software and means were compared using the least significance test at 5% probability level (LSD 0.05%).

Results

Table 2
Analysis of variance of the effect of vernalization and GA_3 on phenology of evening primrose

Source of Variation	Stem Formation	Flower Production	Capsule Filling Time	Side stem formation	physiological Ripening	Maturity	harvest time
GA_3	0.82^{ns}	0.01^{ns}	28.07**	34.99^{ns}	3.24^{ns}	2.38^{ns}	15.28^{ns}
Vernalization	1892.26**	3992.38**	8497.51**	902.83**	9197.3**	10092.17**	18881.83**
GA3 × Vernalization	6.048^{ns}	9.73**	10.71*	534.44**	7.88^{ns}	2.22^{ns}	485.41^{ns}
Error	6.061	2.814	3.105	166.102	3.853	7.343	400.146
CV	6.135	2.873	2.068	54.474	2.209	2.916	15.808

** shows significance at the 0.01 % probability levels; * shows significance at the 0.05 % probability level; ^{ns} shows non-significance.

Table 3
Growth stages from sowing time to stem formation in evening primrose

	Growth stages					
Sowing Date	Germination	4 Leaves	6 Leaves	8 Leaves	10 Leaves	Stem Formation
19 April 2018	26 April 2018	5 May 2018	11 May 2018	21 May 2018	29 May 2018	2 June 2018

Table 4
Effect of vernalization and GA_3 on phenological parameters of evening primrose

Treatments	Stem Formation	Flower Production	Capsule Filling Time	Side Stem Formation	Physiological Ripening	Maturity	Harvest Time
0 days. V	0.000NonS	0.000NonS	0.000NonS	0.000NonS	0.000NonS	0.000NonS	0.000NonS
10 days. V	47.278ab	67.889b	99.500a	23.445ab	102.722b	107.500b	155.667a
20 days. V	48.500a	69.889a	101.500a	17.778b	106.111a	109.000ab	155.000a
30 days. V	46.333ab	68.500ab	101.000a	32.556ab	103.889ab	110.722a	143.278a
40 days. V	45.000b	66.556bc	96.889b	36.223a	101.500b	107.444b	143.944a
50 days. V	48.778a	70.167a	101.500a	32.111ab	105.945a	110.222ab	147.278a
60 days. V	45.000b	65.667c	96.000b	23.500ab	100.444b	105.556b	140.611a
GA_3 0ppm	40.270ns	58.365ns	84.381b	24.572ns	88.381ns	92.683ns	125.937ns
GA_3 500ppm	39.984ns	58.397ns	86.016a	22.746ns	88.937ns	93.159ns	127.143ns

V: vernalization, GA_3: gibberellic acid, NonS: no stem formation, a: maximum time, d: minimum time; means with the similar letters show no significant difference at 5% or 1%.

Application of GA_3 (500 ppm) did not have a significant influence on the phenology of evening primrose. GA_3 was effective only at the capsule filling stage (Table 2). According to the results, cold temperature vernalization had a significant effect on all phenological stages (Table 2). Additionally, the interaction of vernalization and GA_3 on the flower production, side shoot formation, and capsule filling stage were significant (Table 2). On the other hand, their interaction did not significantly influence the stem formation time, physiological ripening stage of the capsule, capsule maturity, and harvest time.

The growth stages including seedling emergence, seedlings with 4-, 6, 8-, and 10-leaf stages, and stem formation time of the plants obtained from seeds vernalized by cold temperature and treated with gibberellic acid are demonstrated in the Table (3).

Effect of vernalization on stem formation

The results of variance analysis (Table 2) showed a significant difference in stem formation of rosette plants of seeds which were vernalized for different periods (10, 20, 30, 40, 50, and 60 days) at 4 °C. Stem formation was observed in all treatments,

and only control plants remained in the rosette stage. There were significant differences among vernalized treatments (Table 4). In the seed samples which were vernalized for 10, 30, 40, and 60 days at 4 °C, the stem formation was observed earlier than the seeds which were vernalized for 20 and 50 days.

Effect of vernalization on flowering time

In agriculture, flowering time is an important factor in production. Both early and late flowering can restrict the plant's normal growth. According to the results of ANOVA (Table 2), vernalized evening primrose seeds had significantly different flowering time that the control plants (P≤0.01). Seed samples, which were vernalized for 40 and 60 days, flowered earlier than the other samples (Table 4). Surprisingly, seeds that were treated for 20 and 50 days produced flowers later than the other groups.

The interaction between vernalization and gibberellic acid (GA_3) had a significant effect on flowering time at a P≤0.01 (Fig. I). The plants of seeds that were vernalized at 4 °C for 30, 40, and 60 days entered the flowering phase earlier than the others. On the other hand, seeds that were vernalized at 4 °C for 10, 20, 40, and 60 days and treated with GA_3 flowered earlier than those vernalized for 30 and 40 days. Vernalization alone had more influence on early flowering than in combination with GA_3.

Effect of vernalization on capsule formation time

The results of the analysis variance of the obtained data showed that vernalization had a significant effect (P≤0.01) on the time of capsule formation in evening primrose (Table 4). Capsule formation of the seeds that were vernalized for 30, 40, and 60 days, occurred earlier than those vernalized for 10, 20, and 50 days.

Effect of vernalization on capsule filling time

The comparison of mean values showed that vernalization had a significant effect (P≤0.01 on the time of capsule filling. In the seeds vernalized at 4 °C for 40 and 60 days, capsules were filled earlier than those vernalized for 10, 20, 30, and 50 days (Table 4). The time of capsule filling was

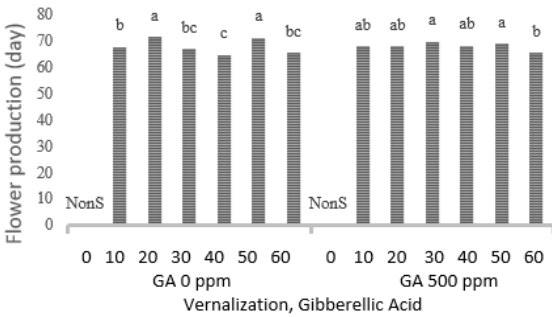

Fig. I. Interaction effect of vernalization & GA_3 on flower production time; NonS: no stem formation, a: maximum time, b: minimum time; means with similar letters are not significantly different at 5% or 1% probability levels.

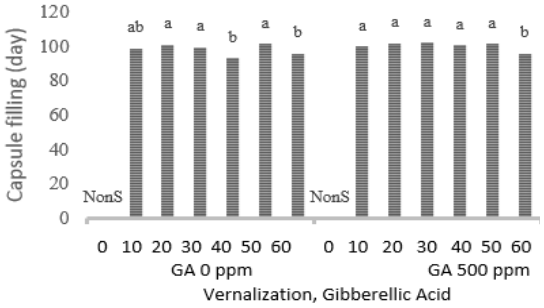

Fig. II. Interaction effect of vernalization and GA_3 on capsule filling time; NonS: no stem formation, a: maximum time, b: minimum time; means with similar letters are not significantly different at 5% or 1% probability levels.

recorded according to the changes in appearance and color of capsules (Fig IV, B). It seems that capsule filling was relatively better in the seeds treated for 40 and 60 days of vernalization at 4 °C.

Statistical analysis showed that applying GA_3 had a significant effect on the capsule filling time at P≤0.01 (Table 4). Non-application of GA_3 as compared to the use of 500 ppm GA_3 resulted in a longer period of time before the capsules were filled. Capsule filling in the plants of seed samples which were vernalized at 4 °C for 10, 40, and 60 days started earlier than that of plants vernalized for 20, 30, and 50 days (Fig. II). The capsule-filling stage of plants of seed samples which were simultaneously vernalized with low temperature (4 °C) for 60 days and treated with GA_3 (500 ppm) started earlier than that of other treatments.

Fig. (II) shows that the combination treatment of GA_3 and cold temperature delayed the seed production of plants. This phenomenon could be

due to the nature of GA_3 as a phyto-hormone. In the plants grown from the seeds vernalized for 40 days, capsules were filled 7 days earlier than those grown from the seeds treated with the same vernalization periods in combination with gibberellic acid (Fig. II). However, there was no significant difference in capsule filling in other treatments.

Effect of vernalization on the time of side stem appearance

Analysis of variances showed that vernalization had a significant effect (P≤0.01) on the time of side stem formation in evening primrose. Comparison of means showed that plants grown from seeds that were vernalized for 20 days at 4 °C produced side branches earlier than those vernalized for 50 days. On the other hand, there was no significant differences among other treatments of the study (Table 4).

The interaction of vernalization and GA_3 application significantly influenced the time of side stem appearance at P≤0.01. In plants cultivated from seed samples vernalized for 20 days, a noteworthy absence of side stems was evident, highlighting a significant distinction compared to the remaining treatments (Fig. III). Also, lateral stems in the plants grown from seeds that were vernalized for 20 and 40 days and treated with GA_3 emerged earlier than those of the seeds vernalized for 10, 30, 50, and 60 days in combination with GA_3 (Fig. III). It seems that by increasing the duration of vernalization treatment to 60 days and in the presence of GA_3 the production of side stems accelerates.

Effect of vernalization on physiological ripening time

According to the results of the analysis of variance, the effect of vernalization was significant on the time of physiological ripening of capsules in evening primrose. Shorter physiological ripening time of capsules were recorded in the plants grown from the seeds vernalized at 4°C for 10, 40, and 60 days in comparison with the seeds vernalized for 20, 30, and 50 days (Table 4). The seed and capsule physiological ripening was determined based on the changes in their

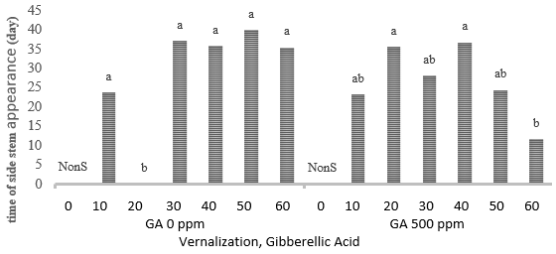

Fig. III. Interaction effect of vernalization and GA_3 on side stem formation time; NonS: no stem formation, a: maximum time, b minimum time; means with similar letters are not significantly different at 5% or 1% probability level

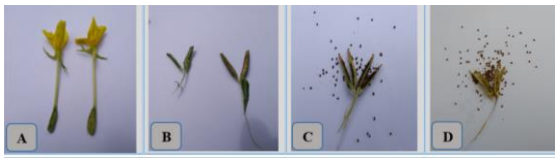

Fig IV. A: flower and capsule formation time, B: capsule filling time, C: physiological ripening time, D: capsule maturity time

appearance and color as well as the hardness of the seeds (Fig IV, C). A more favorable physiological ripening was observed in the seeds treated with cold for 10 and 40 days.

Effect of vernalization on the time of capsule maturity

The results of the analysis of variance showed, that the vernalization effect was significant at P≤0.01 probability level on the time of capsule maturity. The capsule maturity of the plants obtained from the seeds which were vernalized for 10, 20, 40, 50, and 60 days at 4 °C, occurred in a shorter time than the capsules of the plants which were vernalized for 30 days (Table 4).

Effect of vernalization on harvest time

According to the result of comparison means, vernalization had a significant effect on the harvest time in comparison with the control at P≤0.01 probability level (Table 4).

Discussions

In an investigation, Rodrigues and coworkers (Rodrigues et al., 2014) reported that vernalization increased the growth time of wheat spikelets, without affecting flower

fertility. Ramazani and coworkers (Ramazani et al., 2015)demonstrated that wheat seed vernalization negatively affects plant height, number of leaves, and flowering time. Some reserchers (Yadwinder and Dhall, 2017)reported that after two months (8 weeks) of vernalization at 4 °C on 13 genotypes of garlic, flowering was observed in nine genotypes. While the control genotype had vegetative growth and flowering did not observe. A similar result was reported by Fukuda et al (Fukuda et al., 2018)in Onion (*Allium cepa* L.). They found that vernalization caused early flowering in onions before bulblet formation. Ami *et al* (Ami et al., 2013) reported that in onions that had been vernalized for a long time at 5 °C, early stem formation was observed. It has been shown that, by increasing the duration of vernalization at 4 °C, the number of flowering stalks was increased in the *Polianthes tuberosa* plant (Edrisi and Mirzaei, 2017). Keeping garlic bulbs at a low temperature (4 °C) for two months was significantly influenced flower stem production (Yadwinder and Dhall, 2017). It has been proven that the blooming phase of flowering in evening primrose depends on the density of pollen in the first stages of flowering (Anton and Denisow, 2018). Many studies showed that the effect of vernalization is undeniable on flowering and flower formation in different plants. For example, it has been shown that the vernalization of red beet seeds and the Lolium plant at 4 °C for 4 to 12 weeks had a positive effect on flowering (McCormick et al., 2014). Also, vernalization induced early flowering in *Dianthus barbatu* (Dall'Agnese et al. 2014). Compared to the control, early flowering in saffron bulbs was observed when stored at 4 °C for 14 days (Mzabri et al., 2017). The results of another study showed that the vernalization of the wheat seeds at low temperatures resulted in early flowering (Nishiura et al., 2018). Despite that, the plant height and the number of leaves of treated plants were significantly lower than that of non-treated plants (Ramazani et al., 2015). It has been shown that in *Brunonia australis*, when the plant was placed in low-temperature conditions (moist-chilling), the number of flower buds increased from 52 to 72 (Cave et al., 2013). Vernalization accelerates the flowering of wheat in long and short days' conditions (Nishiura et al. 2018). It has been also shown that the vernalization of Raspberry at 6 °C for 6 weeks reduced the number of nodes and increased the flowering period (Sønsteby and Heide, 2012). It was shown that 180 days of vernalization at 4 to 5 °C in combination with GA_3 (600 ppm), caused flowering and increased seed production in onions (Elsiddig et al., 2015). Although treatment of onion with 500 and 1000 ppm GA_3 solution did not have a significant influence on the flowering of this plant, vernalization of onion bulb at 10 °C for 10 days caused early flowering, better growth and seed production (Yalamalle, 2016). It seems that GA_3 is less effective than vernalization at low temperatures (Elsidding et al., 2015).

Turnip seeds vernalized for 40 days promptly entered the flowering phase (Zheng et al., 2018). Khatun and coworkers (Khatun et al., 2016) demonstrated that applying 100 ppm GA_3 for 25 days after seed planting significantly influenced pod number and length, and also seed count. Similar results were observed by El-Din Mekki (Mekki, 2016)with foliar application of 200 ppm GA3 in bean plants. The reason for the lack of similar tendencies in cold-treated seeds for 50 days remains unclear, possibly attributed to seed sample heterogeneity due to evening primrose's indeterminate inflorescence.

The role of gibberellic acid in stem and internode growth has been highlighted (Nagai et al., 2020)). Our findings on evening primrose are in contrast with those reported by Jaques and coworkers (Jaques et al., 2019), where applying GA3 resulted in no significant impact on the physiological quality of chickpea (*Cicer arietinum*). Despite its essential role in growth and development, gibberellic acid's precise accumulation and movement within plants remain unclear (Binenbaum et al., 2018). A plant growth hormone, GA3 synergistically stimulates vegetative growth and delays the reproductive phase when combined with other hormones like auxin and cytokinin. Consequently, GA3 is implicated in prolonging the time to reach the capsule-filling stage. However, GA3's impact on plant reproductive behavior varies among species. Statistical analysis indicated that capsules-filling time was notably influenced by the joint application of cold temperature and GA3. Additionally, while GA3 functions as a flowering

regulator in some biennial plants, it may serve as a flowering inhibitor in perennial species.

Fig. (II) illustrates a non-linear tendency observed in the current parameters. The anticipation was that seeds treated with cold temperatures for 60 days would exhibit improved reproductive growth. The observed results may be attributed to seed sample heterogeneity due to the indeterminate inflorescence of evening primrose plant.

Based on our findings, it is advisable to vernalize evening primrose seeds for at least 10 days at 4 °C. Nonetheless, the effect of vernalization on stem formation varies amongst plant species. The non-consistent response to treatments can be explained considering the indeterminate inflorescence of evening primrose and the utilization of heterogeneous seeds in terms of maturity,

Conclusion

Evening primrose is a biennial plant and needs two consequent growing seasons which most farmers do not welcome it due to the cultivation cost. The results of the present study showed that the vernalization of evening primrose seed using the moist-chilling technique for a minimum of 10 days before planting guarantees the plant flowering in and solving the problem. Although autumn cultivation is used to prevent this limitation, in locations with a moderate winter in most cases some plants still remain in rosette form, which directly influences the final yield of the farm. In areas with a moderate winter, even cultivation in midwinter does not supply the needed cold temperature and plants remain in a rosette form due to the lack of vernalization. On the other hand, in areas with late winter rainfall, field preparation and seed planting is difficult, and delayed cultivation is inevitable. The results of the present research, indicated that flowering occurred in plants obtained from the vernalized seed under vernalization time of 10 or 60 days moist-chilling, even in delayed sowing time in spring. However, in some cases, the combination of the application of GA_3 and vernalization improved some of the parameters. Therefore, it seems that vernalization alone is sufficient to stimulate the flowering of evening primrose in temperate winter areas. It should be noted that the lack of uniformity in observed data is related to the heterogeneity of the used seed samples.

Finally, the result showed that vernalization had a significant influence on the appearance of evening primrose plants at all phenological stages. Therefore, to ensure the seed production, it is strongly recommended that before sowing, the seeds are treated under 2 to 4 ℃ for minimum 10 days.

Acknowledgment

The present work was financially supported by the Deputy of the Science of Gorgan University of Agricultural Science and Natural Resources

References

Ami, E. J., M. T. Islam and A. Farooque. 2013. Effect of vernalization on seed production of onion. *Agriculture, Forestry and Fisheries,* 2, (6) 212-217.

Anton, S. and B. Denisow. 2018. Floral phenology and pollen production in the five nocturnal Oenothera species (Onagraceae). *Acta Agrobotanica,* 71, (2)

Beites, C. L. and L. Morgan. 2014. Evening primrose oil for cervical ripening. *Can J Comp Med,* 13, 47-49.

Binenbaum, J., R. Weinstain and E. Shani. 2018. Gibberellin localization and transport in plants. *Trends in plant science,* 23, (5) 410-421.

Cave, R. L., G. L. Hammer, G. Mclean, C. J. Birch, J. E. Erwin and M. E. Johnston. 2013. Modelling temperature, photoperiod and vernalization responses of Brunonia australis

(Goodeniaceae) and Calandrinia sp.(Portulacaceae) to predict flowering time. *Annals of Botany,* 111, (4) 629-639.

Dall'agnese, L., C. Petry, F. Backes, N. Schwab, L. Girard and R. Bellé. 2014**.** Effects of vernalization on flowering of Dianthus barbatus. *Proc. XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1104*, *2014*:191-196:

Dhall, R. K. 2017. Effect of vernalization on flowering and true seed prodaction behavior of garlic (Allium sativum) under North Indian Plants. *Indian Journal of Agricultural Sciences,* 87, (11) 1554-1558.

Dietz, B. M., A. Hajirahimkhan, T. L. Dunlap and J. L. Bolton. 2016. Botanicals and their bioactive phytochemicals for women's health. *Pharmacological reviews,* 68, (4) 1026-1073.

Dixon, L. E., I. Karsai, T. Kiss, N. M. Adamski, Z. Liu, Y. Ding, V. Allard, S. A. Boden and S. Griffiths. 2019. VERNALIZATION1 controls developmental responses of winter wheat under high ambient temperatures. *Development,* 146, (3) dev172684.

Edrisi, B. and S. Mirzaei. 2017. An investigation into the effect of gibberellic acid and storage temperature on vegetative and reproductive characteristics of tuberose (Polianthes tuberosa). *Journal of Ornamental Plants,* 7, (2) 137-146.

Elsiddig, E. A., O. M. Elamin and M. E. Elkashif. 2015. Induction of flowering in texas early grano onion cultivar using vernalization and gibberellic acid under gezira state Conditions, Sudan.

Fukuda, M., Y. Yanai, Y. Nakano and T. Higashide. 2017. Differences in Vernalization Responses in Onion Cultuvars. *The Journal of Horticulture Science and Biotechnology*. 1-7.

Fukuda, M., Y. Yanai, Y. Nakano and T. Higashide. 2018. Differences in vernalisation responses in onion cultivars. *The Journal of Horticultural Science and Biotechnology,* 93, (3) 316-322.

Ghasemnezhad, A. and B. Honermeier. 2012. Effects of Nitrogen and Pre-Harvest Desiccation on Seed Yield and Oil Quality of Evening Primrose (Oenothera biennis L.). *Journal of Medicinal plants and By-product,* 1, (1) 61-65.

Greiner, S. and K. Köhl. 2014. Growing evening primroses (Oenothera). *Frontiers in plant science,* 5, 38.

Jaques, L., I. R. Carvalho, V. J. Szareski, J. R. Pimentel, C. Troyjack, S. M. Dellagostin, M. T. Mendonça, T. C. D. Rosa, F. A. Villela and V. Q. D. Souza. 2019. Gibberellic acid utilization in seeds and plants of beans: effect on growth and seeds physiological quality. *Journal of Agricultural Science,* 11, (2) 541.

Khatun, S., T. Roy, M. Haque and B. Alamgir. 2016. Effect of plant growth regulators and their time of application on yield attributes and quality of soybean. *International Journal of Plant & Soil Science,* 11, (1) 1-9.

Mccormick, J., R. Goodger and R. Chynoweth. 2014. Cardinal temperatures and vernalisation requirements for a selection of vegetables for seed production. *Agronomy New Zealand,* 44, 71-83.

Mekki, B. 2016. Growth and yield of mungbean (Vigna radiata L.) in response to gibberellic acid and uniconizole foliar application. *Int J Chem Tech Res,* 9, (3) 76-82.

Mzabri, I., M. Legsayer, M. Chetouani, A. Aamar, N. Kouddane, A. Boukroute, I. Bekkouch and A. Berrichi. 2017. Saffron (Crocus sativus L.) yield parameter assessment of abiotic stressed corms stored in low temperature. *Journal of Materials and Environmental Sciences,* 8, (10) 3588-3597.

Nagai, K., Y. Mori, S. Ishikawa, T. Furuta, R. Gamuyao, Y. Niimi, T. Hobo, M. Fukuda, M. Kojima and Y. Takebayashi. 2020. Antagonistic regulation of the gibberellic acid response during stem growth in rice. *Nature,* 584, (7819) 109-114.

Nishiura, A., S. Kitagawa, M. Matsumura, Y. Kazama, T. Abe, N. Mizuno, S. Nasuda and K. Murai. 2018. An early-flowering einkorn wheat mutant with deletions of PHYTOCLOCK 1/LUX ARRHYTHMO and VERNALIZATION 2 exhibits a high level of VERNALIZATION 1 expression induced by vernalization. *Journal of plant physiology,* 222, 28-38.

Ramazani, S. H. R., M. Ebrahimi, H. Ghazvini, M. Jalal Kamali and A. I. Darbandi. 2015. Growth habit and vernalization requirement in some of Iranian bread wheat cultivars.

Rodrigues, O., M. C. C. Teixeira, E. R. Costenaro, L. Vargas and R. Damo. 2014. Influence of Vernalization and Photoperiod on the Duration of Stem Elongation and Spikelet Fertility in Wheat. *Agricultural Sciences,* 5, (14) 1547.

Sohrabi, O., A. Ghasemnezhad, A. Nadimi and M. Shahbazy. 2017. Seed oil quality of GA3 induced flowering evening primrose (Oenothera biennis L.). *Iranian Journal of Plant Physiology,* 7, (2) 1971-1981.

Sønsteby, A. and O. Heide. 2012. Effects of photoperiod and temperature on growth, flowering and fruit yield in annual-fruiting red raspberry cultivars (Rubus idaeus L.). *European Journal of Horticultural Science,* 77, (3) 97.

Timoszuk, M., K. Bielawska and E. Skrzydlewska. 2018. Evening primrose (Oenothera biennis) biological activity dependent on chemical composition. *Antioxidants,* 7, (8) 108.

Woods, D. P., T. S. Ream, F. Bouché, J. Lee, N. Thrower, C. Wilkerson and R. M. Amasino. 2017. Establishment of a vernalization requirement in Brachypodium distachyon requires REPRESSOR OF VERNALIZATION1. *Proceedings of the National Academy of Sciences,* 114, (25) 6623-6628.

Yadwinder, K. and R. Dhall. 2017. Effect of vernalization on flowering and true seed production behaviour of garlic (Allium sativum) under North Indian Plains. *Indian Journal of Agricultural Sciences,* 87, (11) 1554-1558.

Yalamalle, V. 2016. Vernalization Responses in Onion (Allium cepa) Pre-flowering and Reproductive Phases. *International Journal of Bio-resource and Stress Management,* 7, (Dec, 6) 1413-1417.

Zheng, Y., L. Luo, Y. Liu, Y. Yang, C. Wang, X. Kong and Y. Yang. 2018. Effect of vernalization on tuberization and flowering in the Tibetan turnip is associated with changes in the expression of FLC homologues. *Plant diversity,* 40, (2) 50-56.