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REVIEW

Aerial bulbils as garlic alternative planting materials, a systematic review

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ABSTRACT

Garlic (*Allium sativum* L.) is one of the world's most important *Allium* species for various related purposes. The commodity is commonly propagated vegetatively by using garlic cloves. This propagation type has several disadvantages such as a low rate of multiplication, a high need for storage space, persistence of viruses and other infections, and stringent sanitary standards. The accumulation of viruses when using vegetative propagation year after year can reduce garlic yields by up to 50%. As it needs high numbers of cloves, the farmers should spend considerable expenditure on input production for preparing cloves as planting materials. To reduce viral disease problems by using garlic cloves as planting materials, attempts have been established to obtain virus-free garlic cloves through the cleaning of original planting materials by using the meristem cultures. However, this procedure is costly and requires several years to obtain clean planting materials. A potential means to prepare planting materials for garlic is aerial bulbils that can be collected from garlic inflorescence (umbel). The objective of this systemic review is to provide information on an alternative approach to garlic propagation through the use of aerial bulbs or bulbils as planting materials. This systematic literature review describes the garlic flower and aerial bulb morphology, aerial bulbs as plant materials, and environmental factors that possibly promote aerial bulb production. This review hopefully helps the reader to understand the aerial bulbs or bulbils, their characteristics, and their purpose as garlic seeds.

Key words: *Allium sativum*, bulbils, flowering, garlic seeds, inflorescences.

INTRODUCTION

Garlic (*Allium sativum* L.) has an important economic role in the world. Garlic is one of the most important *Allium* species in the world. About 26 million tons of garlic are produced worldwide per year. Garlic is predominantly cultivated in Asia and has gained widespread popularity as a sought-after agricultural commodity among people who prioritize their health (Choi et al., 2021). Unlike onions that are grown by seeds, garlic is mainly vegetatively propagated. All commercial cultivars of garlic are infertile and instead of seeds, garlic flowers contain numerous aerial bulbils that could be used as propagation materials. Naturally, garlic produces bulbils in the stem or scape/topset (Sulastiningsih et al., 2020).

Garlic is commonly vegetatively propagated by using underground bulb cloves. However, this method of propagation comes with a drawback, as it can lead to the transmission of harmful pathogens, viruses, and mycoplasmas. Consequently, this may result in reduced plant vigor, decreased crop yield, and affected overall crop quality. Moreover, it needs high numbers of cloves, the farmers should spend about 60% of their total expenditure on input production, for preparing cloves as planting materials. The continuous use of cloves from the same planting materials has developed virus infections. Celli et al. (2015) reported that garlic affected by the virus showed a 12.3% decrease in weight and a significant 40.2% decline in the number of cloves compared to unaffected, healthy garlic. Multiple viruses belonging to several genera known as the "garlic virus complex" often infect garlic crops. They include potyvirus (*Onion yellow dwarf virus*, OYDV; *Leek yellow stripe virus*, LYSV), carlavirus (*Garlic common latent virus*, GarCLV; *Shallot latent virus*, SLV), tospovirus (*Iris yellow spot virus*, IYSV), allexivirus (*Garlic virus A*, GarV-A; *Garlic virus B*, GarV-B; *Garlic virus C*, GarV-C; *Garlic virus D*, GarV-D and *Garlic virus X*, GarV-X) (Prajapati et al., 2022). According to the findings, a significant reduction in yield can be expected as the years of cultivation and virus exposure in the field pass. This implies that periodic replacement of "seed cloves" with new virus-free material is required to keep the crop profitable.

To reduce the problem of using cloves as planting materials in the vegetative propagation of garlic, an attempt has been carried out by cleaning planting materials free from viruses through the meristem cultures. However, this procedure is costly and requires several years to obtain clean planting materials. Due to the problem in getting the planting materials using cloves that are free from viruses, another alternative of vegetative propagation in garlic is using aerial bulbils. Aerial bulbils or aerial vegetative diaspores could be used for the vegetative propagation of garlic (Zona and Howard, 2022). Growing garlic using aerial bulbils as a form of vegetative reproduction has gained popularity recently. However, in the absence of a specific method for virus eradication, the in vitro propagation from garlic inflorescence or bulbils does not offer any advantage in obtaining virus-free propagation material (Bhusal et al., 2021).

Bulbils, also known as aerial bulbils or top sets, are small structures that closely resemble garlic cloves. They have thin papery skin and are located in the scape of the garlic plant (Chaudhari et al., 2022). However, bulbils require at least one additional year to reach their full bulb size compared to seed cloves. This prolonged maturation period exposes them to potential infection from various pathogens over an extended timeframe. However, if these bulbils are planted in pathogens-free soil, they can offer a cost-effective approach to significantly decrease the infection of propagation material by *Fusarium proliferatum* (Dugan et al., 2019). Using aerial bulbs as planting materials also might reduce the possibility of virus contamination (Godena et al., 2020). Aerial bulbil size is smaller than cloves, and the size and the number of bulbils depend on genotypes (Palani et al., 2014). Several techniques that enhance the production of garlic aerial bulbs are studied. However, a comprehensive review on producing and using aerial bulbs as planting material has yet to be published.

The objective of this systemic review is to provide information on an alternative approach to garlic propagation through the use of aerial bulbs or bulbils as planting materials. This systematic literature review describes flower and aerial bulb morphology, aerial bulbs as plant materials, and environmental factors that possibly promote aerial bulb production.

FLOWER AND AERIAL BULB MORPHOLOGY

Garlic flowers are no different from other *Allium* species. On *Alliums*, flowering genes that are known as *Flowering Locus* T (*FT*) do not only control the flowering initiation but also the bulbing stage of *Alliums*. Moreover, *FLOWERING LOCUS* transcript levels were significantly involved by photoperiod, temperature, and drought (Manoharan et al., 2016). Drought at any reproductive stage significantly decreases the number of florets per umbel (inflorescence) and *Allium* seed production (El Balla et al., 2013).

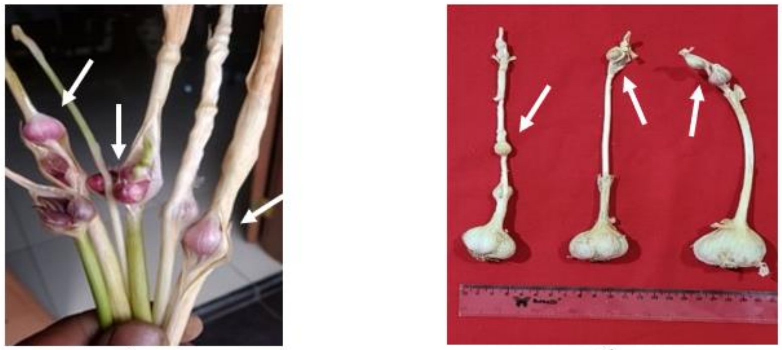
Allium flowers are classified as perfect flowers, consisting of six petals, six anthers, and three locules, each with two seeds. In general, the size of garlic flowers is more petite than shallots, with the number of flowers in the umbel varying from 10-300, typically ranging from 150-200 (Takagi, 2020). Garlic flowers experience pollen release and stigma acceptance ranging from 2-4 d, whereas the stigma can receive pollen ranging from 1-2 d and the anthesis process occurs for 5-20 d. The color of the petals in garlic clones is usually light purple, the anthers are yellow or purple for clonally propagated plants, while those from seeds are light grey and purple. Garlic's physiological age for flowering initiation is seven leaves, and umbels (inflorescence of garlic) were initially covered by a spathe. After the spathe dehiscence, an umbel-like inflorescence was visible and composed of tiny single flowers and numerous bulbils.

Garlic genetics contributes significantly to flowering, where some clones rarely flower in inductive conditions (non-bolting clones), while several other clones can produce flowers easily (bolted clones) (Takagi, 2020). Kamenetsky et al. (2015) reported that advancements in fertility restoration within various genotypes have resulted in successful flowering and seed production in garlic. This breakthrough has opened up new opportunities for genetic research and breeding in the field of garlic cultivation. However, in tropical countries, fertile garlic has not been reported yet. While some imperfect flowering garlic cultivars were reported such as Lumbu Kuning, Lumbu Hijau, Tawangmangu Baru, Batu Malang, Karo, Sembalun, Lokal Bukittinggi, Jatim Lokal 1, Jatim Lokal (Azmi et al., 2022).

Garlic umbels arise from the common meristem. The size of each flower has a distinct morphology that is typical of the *Allium* genus. The elongation of the flower stalk precedes the swelling of the apical meristem and it is divided into several floral developmental centers. In garlic, incomplete bolting clones result in very short scape development, whereas complete threading results in flower induction, scape elongation, inflorescence development, and flower maturation processes (Takagi, 2020). Flower and top set formation are nearly complete when the scape is about 30 cm long, and when it is 35-50 cm, the spathe is exposed. Non-bolting garlic plants will experience the formation of cloves earlier than bolting plants. This shows that there is competition between bulb development and inflorescence, where bulb formation is preferred and flower development is optional. During the growth of garlic flowers, there is balance and competition between the bulb and flower-forming organs in terms of resource allocation where there is a reciprocal relationship between flowers and bulbs in the formation of cloves on bulb and bulbils on inflorescences.

The environment affects the flowering of plants. The timing of flowering in plants is affected by various factors such as nutrients, ambient temperature, drought, salinity, externally applied hormones and chemicals as well as pathogenic microbes. When plants encounter these stress or stimuli, they can respond in two ways: Either by initiating flowering to produce seeds for the next generation or by postponing flowering through a reduction in their metabolic rate (Cho et al., 2017).

Several garlic flower growth and development studies have shown unsuccessful flower competition with bulbils. Garlic seeds can be produced without removing the bulbils, another result found that to enhance flower and seed development, the bulbil should be removed during flower development. This indicates that there is competition between bulbil formation and developing flowers, where this competition will result in flower abortion. In *Allium oleraceum* L., aerial bulbils dominated the flower, with a flower:bulbil ratio less than 0.5 (Fialová et al., 2014). Aerial bulbils were formed because of the reverse effect from the reproductive to the vegetative stage as a result of unfavorable temperatures (Winiarczyk et al., 2014). Aerial bulbils (Figure 1a) arise from the flower stem (tip or middle) or the flower as a failure by the plant to develop flowers/seeds (Azmi et al., 2022) (Figure 1b). Li et al. (2022) reported that aerial bulbils in *Lilium* emerging at lower stems might cause decapitation, while the application of auxin would promote aerial bulbil emergence at upper stems.



1a 1b

Figure 1. Aerial bulbils (a) and aerial bulbil position on the middle and top of flower stalk (b). White arrows indicate the position of aerial bulbils.

AERIAL BULBILS AS PLANTING MATERIALS

Garlic production and propagation are carried out vegetatively, although some garlic clones were found to initiate and develop flowers, such as Central Asian clones (Takagi, 2020). The use of aerial bulbils as planting materials was able to produce garlic bulbs that were equivalent in size to those produced using cloves, while other studies showed that bulbils were very useful in generating bulbs (Dinda and Triharyanto, 2020). Bulbils are less infected by viruses, consequently, they have the utmost potential for the proliferation of healthy bulbs. Another study designated the potential of bulbils to be developed as planting materials in garlic production (Sulastiningsih et al., 2020). Similar results also revealed that bulblets can be used as garlic seeds like cloves (Mhazo et al., 2014; Triharyanto et al., 2022). Because bulbils do not develop in direct contact with soil, they are thought to have a lower pathogen load and are used in garlic propagation.

Growing garlic through the bulbils possibly increases garlic bulb yields and reduces production costs. Moreover, garlic from bulbils showed lower infected pathogen symptoms than garlic from cloves (Dugan et al., 2019). However, garlic bulbils are not free from viruses, and without virus eradication, the bulbils might still contain viruses (Bhusal et al., 2021). A recent study, however, discovered that some viruses also accumulate in the bulbils, indicating the need for additional treatments before bulbils can be used effectively for garlic propagation. A large viral pool was also discovered in the mother plant. Furthermore, Bhusal et al. (2021) reported in their findings that potyviruses can be discovered in the inflorescence meristem of the parent plant and almost definitely appear in completely developed bulbils. Despite the potential of aerial bulbils as planting materials due to the lower incidence of fungi such as *Fusarium* in bulbils as planting materials, recent studies point out that the use of aerial bulbils as planting materials needs to be further studied due to virus accumulation.

Some cultivars of garlic produce many bulbils. In some plants, bulbil phenomena exist, e.g., when the flowering process is halted, and expanding changes generate new plantlets or bulbils rather than flowery organs or seeds but often form along with seeds in a flower bud. The bulbil phenotype is also believed to serve as a spreading mechanism for garlic to conserve itself in nature (Winiarczyk et al., 2018).

The use of bulbils in garlic cultivation has been developed in Indonesia and other countries such as South Korea, India, and Zimbabwe. In several regions in Indonesia, farmers have started using bulbil seeds to produce garlic clove. A study was reported from Ladakh, Himalaya, India. They obtained that pea-size bulbils planted in September before the beginning of severe winter form typical plants bearing one or two cloves bulbs with a weight of a maximum of 28.6 g within turn produced normal plants carrying many cloves and bulbils in the second years of cultivation. In comparison, the Indonesian yield was lower around 15 g per plant (Triharyanto et al., 2022).

Using aerial bulbils as a planting material results in the production of variations of garlic cloves. Various yields have been obtained from various studies using aerial bulbils as planting materials. Sometimes bulbil plants resulted in bulbs and cloves being smaller than normal cloves. In addition, the application of mulch can stabilize the temperature of the soil and perform better results on the growth and yield of garlic using bulbils (Dinda and Triharyanto, 2020). Moreover, aerial bulbils have a dormant period that prevents them from germinating. Low temperatures could break the dormant period, affect the endogen hormone, and help the aerial bulbils germinate (Dong et al., 2019).

ENVIRONMENTAL MODIFICATION TO PROMOTE AERIAL BULBILS

The most important variables to enter the generative phase are photoperiod and temperature (Sopha et al., 2014). Photoperiod is recognized mostly by developed leaves in whole plants. Temperature, especially vernalization (low temperature) is noticed by the shoot apex, and water availability is distinguished by the root system. The tropical region has a warm temperature (on average above 20 °C) and a short photoperiod, no more than 12 h a day. While *Alliums* need low temperatures and long photoperiods (Sopha et al., 2014), environmental modification is required to promote the generative phase of *Alliums* in the tropic region.

Appropriate environmental conditions in stimulating flower development will result in varying rates and degrees of flower and bulbil development among flowering garlic clones. Production of viable gametes and seeds is impossible without developing mature flowers. Genetics is one of the main factors affecting garlic's fertility, therefore fertile clones will produce high quantities and quality flowers and small bulbs. Flowering clones are sterile and infertile, affecting the development of flowers and bulbils. The formation of large bulbils can potentially result in low seed production. In general, sterile clones develop flowers that undergo meiosis before buds so that mature flowers do not develop. Some cases of developing flowers will suffer multiple floral abortions during scape elongation in fertile clones.

In overview, the impact of environmental factors, phytohormones, and other bioactive substances on garlic bulbils can be seen in Table 1.

Table 1. Effect of environmental factors, phytohormones, and other bioactive substances on garlic bulbils.

Nr	Factors	Impacts on aerial bulbils and inflorescence	References
1	Temperature	Consistent exposure to warm temperatures during the vegetative and generative phases led to inadequate flowering and failure of sexual organs	Mayer et al. (2015)
		Low-temperature treatment breaks the dormancy of aerial bulbs and increases the production of virus-free seedlings and the quality of garlic	Dong et al. (2019; 2020; 2022)
2	Soil humidity	Garlic bulbils grow better with the use of 100% organic fertilizer and plastic mulch. Plastic mulch can stimulate the increase of plant height, number of leaves, and diameter of the garlic stems	Dinda and Triharyanto (2020)
3	Phytohormones	The best media for shoot induction from the callus of garlic bulbils was MS medium supplemented with 0.1 ppm naphthalene acetic acid (NAA) + 1 ppm benzylaminopurine (BAP) at 16 h photoperiod	Kristina et al. (2020)
		Atonik increases the number of bulbils	Sulastiningsih et al. (2020)
		Gibberellin (GA_3) increases the germination of aerial garlic bulbs by breaking their dormancy	Dong et al. (2019; 2020)
		Gibberellin (GA_3) + cytokinin combined with N $+K_2O$ promoted garlic's bulb production	Samy and El-Zohiri (2021)
4	Bioactive substances	The bioactive profile of garlic grown from air bulbils is dependent on harvesting time, and the concentration of bioactive diminishes with plant maturity	Kopec et al. (2020)

Vernalization

Flowering is triggered by vernalization with arranged gibberellin (GA_3) and inhibitor balance. Bulblets are produced because of vernalization. The highest significant yield was obtained by growing the garlic cloves at 5 °C for 20 d. This result provides strong evidence for the potential cultivation of garlic using various cultivars in various climates or seasons and making off-season is also possible (Wu et al., 2015). In Guarapuava, Paraná State, Brazil, the use of 40 d vernalization periods resulted in better adaptation of garlic to photoperiod and temperature, resulting in good vegetative and productive development. Furthermore, after vernalization at 4 °C, certain garlic genotypes developed seeds and entered the bolting phase (Yadwinder and Dhall, 2017). Vernalization could boost the production of plant growth regulators and cause the garlic to bolt (Santos et al., 2022).

Fundamental physiological research states that environmental manipulation can produce flowering and fertility restoration in garlic. Exposure to low temperatures (5 °C) is required before the start of the major growth period in garlic to trigger the development of the apical meristem of the flower. Takagi (2020) observed, however, that exposure to low temperatures, especially high ones, results in inadequate defoliation during the inflorescence phase and scape elongation. Mayer et al. (2015) found that temperature regulation occurs in garlic at several stages of florogenesis and that high temperatures can limit pollen formation, fertilization capacity, and seed generation. The gradual rise in temperature before and during anthesis encourages the growth of intact blooms in both fertile and sterile male plants. Transplanting bolting plants from 19 to 31 °C produces spathe breaks 10-20 d earlier, better antheses, shorter, dense inflorescences (300-400), and only a few small top sets. Meanwhile, chronic 19 °C exposure causes poor blooming, anther abortion, and decreased pollen output. The most susceptible phase of microsporogenesis in both sterile and fertile male plants is the unicellular microspore stage. Khodorova and Boitel-Conti (2013) stated that the temperature of the early stages of flower organogenesis varies, between 9 and 25 °C followed by lower temperatures of 4-9 °C for several weeks, it will be possible to produce stem elongation and flowering. According to Ben Michael et al. (2018), applying garlic vernalization treatment at 4 °C for 4 and 6 wk would result in the greatest number of flowers per umbel 200-240, and bolting (Wu et al., 2015; 2016).

Photoperiod

Common *Alliums* need a long day and vernalization to get flowering. The flowering of tropical shallots was improved by a long day as well as of garlic and subtropical onions (Sopha et al., 2014). A longer photoperiod than usual can result in faster and simultaneous flowering.

Exposure to adequate environmental stimuli during pre-planting storage and germination before the following growth stage has a substantial influence on garlic florogenesis. Genetic and environmental variables have a significant role in floral stalk elongation. The long photoperiod affects stalk elongation in the bolting genotype, with a long photoperiod (16 h) of 10 d resulting in a long flower stalk (30 cm) and a higher number of flowers per umbel than natural irradiation. However, a long photoperiod (16 h) of 30 d results in a slowdown in the length of the flower stalk and an early abortion of the developing inflorescence bud. Furthermore, bulbil yield was positively linked with plant tallness, flower stalk length, and the number of leaves per plant.

Plant growth regulator

Gibberellin (GA_3) increases the germination of aerial garlic bulbs by breaking their dormancy (Dong et al., 2019; 2020). The number of cloves per bulb rises whenever garlic plants get treated with gibberellin solution. Flowering and bulb formation in garlic are controlled by unique FT genes (*FLOWERING LOCUS* T), like in onions (Khokhar, 2022).

The effect of other plant growth regulators on bulbils was reported. Sulastiningsih et al. (2020) reported that soaking bulb seeds in 3 mL L^{-1} Atonik (PT Oat Mitoku Agrio, Indonesia) for 4 h increased the number of bulbils 4 mo after planting bulbs (Figure 2). Sodium ortho nitrophenol, sodium para nitrophenol, sodium 2,4-dinitrophenol, indole butyric acid (IBA) (0.057%), and 5-nitro guaiacol sodium salt are all present in Atonik, a non-synthetic growth regulator. Atonik seems to play an important role in enhancing the cell wall permeability of treated bulbils which will boost and quicken the absorption of nutrient elements, and then accelerate the construction of chlorophyll to support the formation of new bulbils. Samy and El-Zohiri (2021) confirmed that a combination of approved N and K fertilizers (up to 180 kg N + 92 kg K_2O), with 100 ppm GA_3 and 2.5 ppm cytokinin, promoted garlic's bulb production.

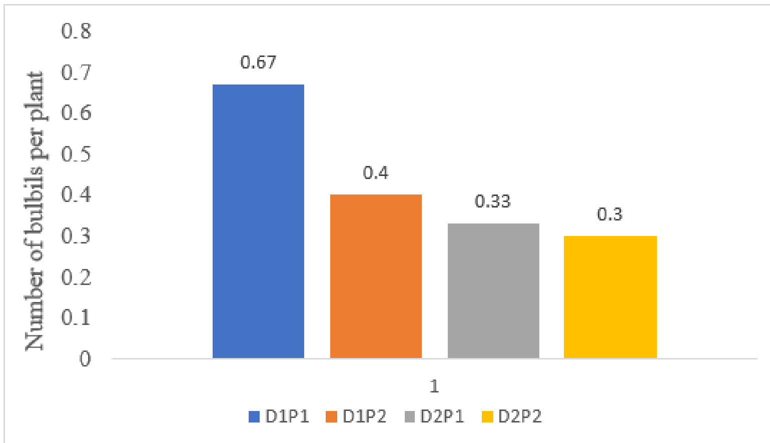


Figure 2. Average number of bulbils produced applying non-synthetic growth regulator Atonik. Immersion of bulbils in Atonik solution: 3 mL L^{-1} for 4 h (D1P1) and for 8 h (D1P2), 6 mL L^{-1} for 4 h (D2P1) and 8 h (D2P2) (Source: Sulastiningsih et al., 2020).

CONCLUSIONS

Aerial bulbils are potentially alternative planting materials for growing garlic. Although employing those planting materials would not eliminate garlic viruses, it will probably minimize the incidence of the pathogen and enhance plant health, resulting in a higher yield. Vernalization, photoperiod, and plant growth regulator treatments are three environmental adjustments that could be explored to increase aerial bulbil production. Furthermore, tissue culture of aerial bulbils to eliminate viruses could be advantageous. Increased day length and GA_3 treatment could break the dormancy of aerial bulbils. Research activities that should be established include micropropagation of aerial bulbils to eliminate the virus complex, breeding for obtaining new garlic varieties resistant to virus diseases, and improved techniques on garlic cultivation using aerial bulbils.

Author contribution

Conceptualization: G.A.S. Methodology: All Authors. Formal analysis: All Authors. Investigation: All Authors. Resources: All Authors. Data Curation: All Authors. Writing-original draft: G.A.S., S.F.S., I.C., A.E.M., K.K.H., N.G., I.P., Y.H. Writing review and editing: G.A.S., S.F.S., M.P.Y., A.M. Visualization: All Authors. Supervision: M.P.Y., S.F.S., A.M. Project administration: All Authors. Funding acquisition: A., D.P., Y., S.E. All co-authors reviewed the final version and approved the manuscript before submission.

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