INTRODUCTION

Drought is a natural phenomenon that greatly influences life on earth. Drought can be defined as an area with a below-average rainfall duration compared to the average rainfall of that area at any time of the year [1]. Two-thirds of the earth's human population (equivalent to four billion people) live with water scarcity [2]. Drought is a situation in which the lack of water availability in an area is used to meet needs for a relatively long period of time [3]. Drought is also related to high air temperatures during winter due to below-average water repositories. These situations are usually found in developing countries that experience winter [4]. It also significantly impacts the drought that occurs on wheat fields.

Wheat is one of the staple food crops that supply 20% of calories globally [5; 6]. Wheat is a major ingredient in manufacturing foods such as...
Wheat crops are especially sensitive to heat stress and drought at the stages of flowering and grain development, which negatively impacts grain yield and quality [7]. In drought conditions, the growth rate of wheat roots is reduced [8]. Drought can also reduce leaf area, reducing photosynthesis as a consequence. In addition, the number, size and age of the leaves, and the age of the leaves can be shrunk due to water stress [9]. Wheat that is experiencing drought tends to produce thin wheat grains and reduce the amount of harvest compared to when water conditions are met and sufficient for wheat harvesting [10].

Wheat demand is expected to increase by 60% in 2050, but production may decrease by 29% because of climate change impacted by environmental pressures [11]. These predictions suggest that increasing abiotic stress tolerance in wheat will be critical to global food security soon. Therefore, sustainable genetic improvement of wheat is essential because it has a direct impact on economic development, food security, and international grain trade.

Water greatly affects the productivity of wheat crops. To increase wheat crop productivity, drought-tolerant wheat crops should be utilized. It is imperative to ensure food security in times of drought. The development of drought-tolerant wheat crops may prevent the unnecessary expansion of wheat fields due to the stable and sufficient productivity of wheat when drought occurs.

Fortunately, the development of knowledge and technology now allows mankind to identify the main regulator of drought tolerance in wheat crops using a Genetically Modified Organism (GMO) crop approach. Drought-resistant wheat crops are made through genetic engineering by transferring drought-tolerant cells to wheat crops [12]. Wheat with drought-tolerant properties is a genetically modified crop, obtained through the insertion of cold shock protein genes that have increased adaptability to a low-water environment [13]. Cold shock proteins are composed of nucleic acids that will be attached to strands of Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA). RNA molecules tend to form stable secondary structures at low temperatures, resulting in translational functions and DNA transcription is not working properly. Therefore, a cold shock protein should be utilized to maintain the instability of the RNA form at low temperatures so that translation and transcription can still occur at low temperatures [14]. The process requires vectors by Agrobacterium to transfer genes in wheat crops [15]. The method of genetic transformation using Agrobacterium vectors is done by injecting the bacteria into the plant to insert recombinant plasmids in plant cells so that the plant carries a needed property. The particle bombardment method is done by biolistic tools or gene shots by coating the gene of interest that has been inserted on plasmids with gold particles and penetrated on embryonic cells so that it can penetrate the plasma membrane and nuclear membrane. This method succeeds in producing transgenic plants [16]. The application of biotechnology and microbiology plant innovations with drought-tolerant properties provides solutions to improve the quality and yield of wheat crops.

This article aims to provide information related to the insertion of cold-shock genes from *Escherichia coli* in wheat plants to produce drought-tolerant properties that can be studied clearly and correctly. The focus of the study includes drought on wheat fields, wheat crops with drought-tolerant properties, the structure and function of *Escherichia coli* genes that express cold shock proteins, the mechanism of insertion of cold shock protein genes, and the influence of cold shock protein genes on wheat plants with drought-tolerant properties.

**METHODS**

This paper was compiled using research methods of literature review. The literature review was a research method that utilized books, journals, and related literature to solve a problem. The advantage of this method was that the data obtained had been recognized and received, found numerous sources at a cheaper cost, and could be used as a good reflection material. The material used in this study was a state-of-the-art journal related to the problems in this study. The journals used were searched through Google Scholar as well as from other journal sites. Moreover, the journals used were taken from international and national levels to ensure the validity of the data. This type of research was descriptive qualitative research.

**RESULTS AND DISCUSSION**

**Drought on wheat fields.** Almost all areas had experienced drought [3]. Drought is a condition in which the lack of water availability in a particular region occurs for a long time. Since water was usually used for a wide variety of activities, groups, and even environmental sectors, droughts exert a negative impact on living things. In addition to dry season occurrences, droughts could also occur in the winter. Such drought might occur when the temperature was low and the water was frozen [17].
Therefore, it could be said that winter droughts were caused by the freezing of bodies of water, resulting in a lack of water (Figure 1).

Drought could be reviewed based on several aspects, including hydrometeorology, agriculture, and hydrology [18]. Based on agricultural aspects, an area was declared to be experiencing drought if the soil was reduced so that the soil experiences water shortages [18]. From an agricultural aspect, drought would impact crop production, damage crop quality, decrease the income for farmers due to reduced crop yields, reduce the productivity of agricultural land, increase irrigation costs, and impose new costs for the development of additional water resources (wells, dams, pipelines) [19]. Severe drought can reduce crop yields because water and soil moisture limitations inhibit plant growth [20]. Thus, drought could be categorized as one of the most severe natural disasters that cause environmental constraints that limit crop growth, development, and crop yields with tremendous economic and social impacts [21].

Various agricultural lands, including wheat fields, were expected to suffer from losses when hit by drought. Deficits caused by drought significantly reduce crop yields due to their negative impact on crop growth. This is because wheat is a plant that is very dependent on the availability of water in its growth phase. Wheat production is greatly influenced by water availability during the process of wheat growth [22]. Wheat production will exhibit optimal results if it gets enough irrigation, especially in the phase of clump formation, flowering, and filling of grains. During drought, photosynthesis of the pulp will be carried out under less optimal conditions. Lack of water in the pollination phase will result in disruption of the pollination process and a reduced number of seeds. Meanwhile, in the phase of sapling formation and flowering, the formation of juice will be disrupted and result in a decreased number of wheat saplings [22]. Indirectly, drought will also interfere with the roots of the plant. As stated by Fukai & Boonjung in Zhang et al. [23], wheat is sensitive to drought during the vegetative phase because drought limits the development of the rooting system. This results in a decrease in leaf area, number of leaves per plant, leaf size, and leafage. Worse, plants may die if drought occurs for a long time [20].

**Wheat crops with drought-tolerant properties.** Abiotic checks such as drought are factors that greatly influence the development and innovation of GMO crops to address these problems [24]. The increasing number of genetically modified plants that have the ability to withstand abiotic checks continues to be done. An example of its product would be wheat plants with drought-tolerant properties. Wheat with drought-tolerant properties is a genetically modified crop obtained through the insertion of cold shock protein genes from *Escherichia coli* with improved adaptability to a low-water environment [13]. Wheat crops with drought-tolerant properties have lower malondiadehyde (MDA) content, lower water loss rates, relatively lower Na+ content, and higher chlorophyll and proline content than wheat plant control under drought stress. GMO wheat crops increase germination rates, survival rates, and increase in long primary roots compared to organic plants [25]. In addition, wheat crops with drought-tolerant properties can improve the quality of wheat and the quantity of its production [26].

To test the difference between organic wheat and GMO wheat, Yu et al. [13] conducted a study with wheat germ aged seven days left exposed to severe water deficits. After one week, the control plant showed withering and serious death. After a week of rewatering, 97.6% and 93.3% of transgenic plants recovered and grew normally, while only 29.2% of control plants survived and

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**Figure 1.** Winter drought in the wheat field. Source: [41]
grew normally. Additionally, research was also conducted by Wang et al. [27], which showed morphological differences between GMO wheat and wild plants experiencing drought, heat, and its combination.

**The structure and function of Escherichia coli genes that express cold shock proteins (CPSs).** Genes are the forming components of chromosomes. Chromosomes were individuals formed by a set of genes [28]. In the process of expression of cold shock protein in *Escherichia coli*, the process was greatly affected by temperature. *Escherichia coli* bacteria could respond at temperatures above 40 and below 20. The protein responsible for this was cold shock protein. This protein was referred to as CspA protein which induces 13% of cellular protein synthesis at temperatures below 37 [29]. Cold shock proteins were composed of nucleic acids that will be attached to strands of DNA or RNA. RNA molecules tend to form stable secondary structures at low temperatures, resulting in translational functions and DNA transcription not working properly. Therefore, cold shock proteins are needed to ensure and maintain the formation of RNA at low temperatures in order for translation and transcription to also occur at low temperatures [14].

Cold shock proteins (CPSs) are composed of one nucleic acid attachment domain (about 70 residual amino acids) referred to as the cold shock domain (CSD) [14]. CSD also has a structure, which helps in the process of attachment to DNA. CSD has a very important role in the attachment of DNA or RNA strands. The attachment of DNA or RNA strands serves to help the process of gene transcription. CSD is composed of 5 barrels containing the binding of the RNA 000..that contributes to the attachment of one strand of DNA or RNA [14].

**Mechanism of insertion of cold shock protein genes.** The process of genetic engineering to obtain varieties that have the desired properties consists of several techniques, one of which is recombinant DNA techniques. Recombinant DNA is the insertion of a DNA molecule into a vector that forms a new DNA recombinant so that it multiplies in a new host cell [30]. The insertion of DNA molecules is used to take a useful trait from a different type of organism and is tested on the intended organism. DNA technology has the advantage of being able to produce superior properties quickly over artificial selection. According to Klug and Cummings in Mahrus [31], genetic engineering techniques are generally done...
with several steps: isolating, cutting, and transferring certain DNA to target genes. Bacterial cold shock proteins are multifunctional DNA/RNA binding proteins characterized by the presence of one or more cold shock regions [32]. This genome can be found in several bacteria, one of which is *Escherichia coli*. These bacteria contain nine genes analogous to the CspA gene, four of which are CspA, CspB, CspG, and CspI [14]. At low temperatures, gene transcription will increase as protein translation is enhanced through the elimination of stabilized RNA secondary structures [13]. Thus, the CspA gene can help bacteria survive despite low temperature conditions.

Low temperatures significantly impact living things because many proteins and enzymes are unable to work at cold temperatures. In agriculture, the advantages of these gene-expressed traits greatly help agricultural crops to survive in winter to maintain production in impossible conditions. One of the growing fields of agriculture in subtropical regions is wheat that cannot survive during winter. Genetic engineering by inserting cold shock protein genes could bring hope to the wheat farming industry in the future.

According to Yu et al. [13], the insertion of cold shock protein genes into plant cells needs to be modified to improve gene expression by altering nucleotides in DNA sequences to increase the content of G+C. This is done to increase the genes that are generally present in plants without changing the amino acids of CspA and CspB, modifying these genes called SeCspA and SeCspB. In research Yu et al. [13], the SeCspA and SeCspB genes are combined in the pAHC25 vector with the ubiquitin control promoter (Ubi), and the bar is used as a gene selection marker in plants. Amorphous cells from KNI99 wheat embryos (winter wheat) are cultured at 26 with dark conditions to be used in the transformation process by the microprojectile bombardment method. This method is a shot of particles that is done physically with a “gene gun”, after which the amorphous cell is incusted for four weeks with Bialaphos media until the plantlet grows and then transferred on a plantlet reinforcement medium to grow roots [13]. Plants that have been rooted are transferred to pots and grown in greenhouses. GMO wheat is then tested with PCR techniques to determine the success of this process.

**Effect of cold shock protein genes on wheat crops on drought tolerant properties.** Biotechnology has grown rapidly over the years and produces many technologies that help the development of science. Genetic engineering methods have also developed at a rapid rate. For example, microprojectile bombardment methods or gene shots have just been recently innovated. This method is a very promising alternative to genetic transformation in plants as it overcame the limitations of the genetic transformation method with Agrobacterium [34]. The method of genetic transformation using *Agrobacterium* vectors is done by injecting the bacteria into the plant to insert recombinant plasmids in plant cells so that the plant carries the desired property. The particle bombardment method is done by biolistic tools or gene shots by coating the gene of interest that has been inserted on plasmids with gold particles and fired on embryonic cells so that it can penetrate the plasma membrane and nuclear membrane. This method succeeds in producing transgenic plants [16]. The disadvantage of this method is that inserted genes may not be expressed or even silenced, while the advantages are that various genes with different lengths can be inserted easily, and this method is vector-less [35]. This study used microorganisms in the form of *E. coli* bacteria. In genetic engineering, *E. coli* are the most common bacteria utilized as hosts for recombinant processes [36]. Advantages of *E. coli* as hosts include their rapid growth, cheaper production costs, and protein expression levels [37]. Another advantage of this bacterium is that it can maintain its growth in a balanced manner at a temperature of about 10 to 49°C. Inside *E. coli* are nine homologous CspA, named CspA to CspI, of similar size and corresponding genes spread across the *E. coli* chromosome [38]. However, among the nine members, only CspA, CspB, CspG, and CspI were reported to have cold shock proteins [39]. The presence of these proteins allowed bacteria to live in both cold and low temperatures.

The existence of this technology certainly has a positive impact, especially for the plant itself. According to research conducted by Yu et al. [13], wheat plants became more resistant to drought than regular wheat crops after receiving a CSP gene insertion. The gene insertion produced a DNA recombinant with specific nucleotide changes. Nucleotide changes were made to improve the overall content of G+C amino acids without changing the existing sequence of both CspA and CspB proteins. These changes play a significant role in helping wheat adapt to drought. Leaves on GMO wheat plants show decreased water loss rates as the stomata on these plants close faster than regular wheat plants. This certainly resulted in GMO wheat crops becoming more resistant to water deficits. In addition to being resistant to drought, Yu et al. [13] reported that GMO wheat crops exhibited more stress-tolerant characteristics and improved physiological properties. The results of the study found that GMO wheat crops did not show a significant increase in grain yields.

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compared to regular wheat crops. Furthermore, GMO products are still not widely accepted among the general public. GMOs remain controversial, both in the product's home country and in the country of GMO users. In fact, controversy over GMO products invites unending scientific inquiries from the sharpest scientists who have their respective opinions [30].

Water droughts occur not only in summer but also during winter. The problem of drought in winter in subtropical regions hit many such areas recently in China in 2017–2018. Winter drought is defined as a state of water shortage at temperatures below 0°C so that water is stored as snow and ice [40]. Winter drought is one of the focuses of research in the field of extreme climate change in the world because it greatly affects agricultural land. Wheat land became one of the lands affected by drought due to winter; therefore, this study was conducted. The presence of drought-resistant wheat can maintain or even increase crop yields amid extreme conditions such as winter. According to Yu et al. [13], the transgenic wheat tested successfully survives drought stress and produces better wheat in terms of drought tolerance than the control variables (non-GMO). For instance, GMO wheat presented increased germination rates, survival rates, and primary root length. The results of this study can contribute a large influence on agriculture so that drought no longer damages agricultural land and harms farmers. This solution can also help the economic growth of an area.

CONCLUSION

Drought is a threat to plants, including wheat. The genes (CSP) of *Escherichia coli* are inserted in plants in order to express genes by altering nucleotides in DNA sequences to increase the G+C content, thereby increasing the genes present in plants without changing the amino acids CspA and CspB, the modifications being called SeCspA and SeCspB. The insertion of these genes makes plants more drought resistant.

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