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***In Silico* mapping of some genes responsible for abiotic stress tolerance in cereals.**

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ABSTRACT

Abiotic stresses such as drought, high salinity, cold and low temperature cause great reduction in yields of major crops and affect plant growth. Modern agriculture faces abiotic stresses, especially salinity and drought as major factors limiting crop productivity worldwide. Response to abiotic stresses is a very complex phenomenon. Plants usually respond to the abiotic stresses at the molecular, physiological and biochemical levels, helping them to adapt with these stresses. MYB transcription factor is one of the major families of plant-specific TFs, has been related to drought and salinity responses. In this review the genes related to MYB TF family are selected and *In Silico* comparative mapping for these genes are performed. The identification and characterization of various genes involved in drought and salinity stress responses play an essential role in crop improvement, such as marker-assisted selection (MAS) and that very important for the breeders, helping them in their breeding programs as well as to develop stress tolerant crops.

Keywords: Drought and Salt stress, Molecular biology, Transcription factors (TFs), Rice genome, Cereals, Functional Genomics, *In Silico* comparative map.

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INTRODUCTION

World population is increasing at an alarming rate and is expected to reach about six billion by the end of year 2050. On the other hand food productivity is decreasing due to the effect of various abiotic stresses. Abiotic stresses cause losses worth hundreds of million dollars each year due to reduction in crop productivity and crop failure; therefore minimizing these losses is a major area of concern for all nations to cope with the increasing food requirements ⁽¹⁾.

Among abiotic stresses, drought and salinity together affect more than 10% of arable land, resulting in a more than 50% decline in the average yields of major crops worldwide ⁽²⁾.

Cereals are staple foods. Cereals are important sources of nutrients, they have a long history of use by humans in both developed and developing countries. Cereals and cereal products are an essential source of energy, carbohydrate, protein, fats and fibre, as well as containing a range of micronutrients such as vitamin E, some of the B vitamins, zinc and magnesium. Also, by the regular consumption of wholegrains of cereals, has a role in the prevention of chronic diseases such as coronary heart disease, diabetes and colorectal cancer ⁽³⁾. Egyptian Cabinet and Information and Decision Support Center (IDSC) suggestion, to solve the food problem in Egypt, was to focus on the agricultural research to enhance the capability of crop plants to withstand different abiotic stresses, such as drought, salinity, cold and heat shock which will lead to higher yields by either increasing the crop set and/or by extending crop cultivation in the areas previously denied due to abiotic stresses ⁽⁴⁾.

In recent years, there is a great interesting in crop response to abiotic stresses because of severe losses that result from drought, salinity, heat and cold stress. An analysis of major world crops shows that there is a large genetic potential for yield that is unrealized because of the need for better adaptation of the plants to the environments in which they are grown ⁽⁵⁾.

Abiotic stresses trigger a series of plant responses, including a number of physiological, biochemical, and molecular responses ⁽⁶⁾. Physiological and biochemical modifications in plants including leaf wilting, reduction in leaf area, leaf abscission, stimulation of root growth, changes in relative water content (RWC), electrolytic leakage (EL), generation of reactive oxygen species (ROS), and accumulation of free radicals which disrupt cellular homeostasis by reacting with lipids, proteins, pigments, and nucleic acids resulting in lipid peroxidation (LP), membrane damage, and the inactivation of enzymes, thus affecting cell viability ⁽⁷⁾. Besides this, abscisic acid (ABA), it is an important plant hormone that plays a regulatory role in many physiological processes in plants, such as regulation of stomatal aperture, and the activation of stress-responsive genes ⁽⁸⁾. Increased levels of ABA are triggered by a variety of environmental stresses such as drought, water stress, salinity, cold, desiccation, heat stress, and wounding. Further, it is also proved that ABA is a major physiological signal that induces drought and high salinity responses ^(9,10,11,12,13). Molecular responses including stress perception, signal transduction to cellular components, gene expression, and, finally, metabolic changes imparting stress tolerance ⁽¹⁴⁾. The genes thus induced by abiotic stresses can be classified into two groups ^(15,16,17). One group constitutes genes that encode proteins to protect the cells from the effects of water stress, such as the genes that govern the accumulation of compatible solutes (key enzymes for osmolyte biosynthesis like proline, betaine, sugars, etc.) and other proteins for the protection of macromolecules [LEA (late embryogenesis abundant) protein, osmotin, antifreeze proteins, chaperons, etc.]. It has been suggested that introduction or over-expression of genes encoding LEA proteins, proline synthetase or betaine synthetase, etc. can provide tolerance to drought or high salinity in transgenic plants ⁽¹⁸⁾. The second group of genes comprises regulatory proteins that further regulate stress signal transduction and modulate gene expression and, hence, probably function in the stress response. They include various transcription factors (TFs) suggesting the role of various transcriptional regulatory mechanisms in the stress signal transduction pathways ^(14, 19).

Transcription factors (TFs) are proteins with a DNA domain that interact with the *cis*-acting elements present in the promoter of a target gene. They induce (activators) or repress (repressors) the activity of the RNA polymerase, thus regulating gene expression. TFs can be grouped into families according to their DNA-binding domain ⁽²⁰⁾. A group of genes controlled by a certain type of TF is known as a regulon. In the plant response to abiotic stresses, at least four different regulons can be identified: (1) the CBF/DREB regulon; (2) the NAC (NAM, ATAF and CUC) and ZF-HD (zinc-finger homeodomain) regulon which are ABA independent regulons; (3) the AREB/ABF (ABA-responsive element-binding protein/ ABA-binding factor) regulon; and (4) the

MYC (myelocytomatosis oncogene)/MYB (myeloblastosis oncogene) regulon which are ABA dependent regulons ⁽²¹⁾, thus imparting stress tolerance ⁽⁸⁾.

According to a recent review reported on structure, function and networks of the transcription factors involved in abiotic stress responses showed that, the transcription factors (TFs) are key regulators of both ABA-dependent and ABA-independent abiotic stress responses ⁽²²⁾. Besides this, drought and salinity stress tolerance and adaptation in rice plants have been improved by engineering various transcription factors, genes related to signalling pathway, compatible solutes and accumulation of antioxidants. The importance of many genes related to several pathways has been clearly elucidated in a review of stress tolerance mechanisms and use of transgenic technology in agriculture for developing abiotic stress tolerant crop plants ⁽²³⁾. So, it is very important to evaluate the exact role of specific transcription factor(s) or gene(s) and then genetic manipulation for the crop improvement ⁽²⁴⁾.

The objective of this study was to identify the drought and salt tolerant genes by using *In-Silico* comparative map, this comparison between the rice genome region responsible for drought and salt, and the other cereals. This research will open a door to new approaches and applications for understanding the mechanisms by which plants adapt to abiotic stress, and should ultimately result in the production of new and improved stress-tolerant crops.

MATERIALS AND METHODS

In silico comparative mapping

The genes of MYB TF family were searched through GenBank databases. The software found in the gramene website (<http://www.gramene.org>), and required accession numbers of the targeted genes, which could be accessed from the nucleotide database. This website carries alignments of genomes of the cereal family. The results obtained from this stage were used to construct a comparative map between rice and maize to identify the tentative chromosomal location of the gene under study using comparative mapping strategy ^(5,25).

Table (1): Genes related to MYB transcription factor (TF) family.

	Genes	TF family	Species	Accession number	Sequence	Reference
1	MYBAS1	MYB	<i>Zea mays</i>	NM_001155730	1398 bp	(26)
2	MYBAS2					
3	OS01G0176700					
4	OS02G0700300					
5	OS09G0431300					
6	OS02G0685200					
7	OS01G0863300					
8	OS04G0583900					
9	OS04G0600000					
10	OS02G0241200					
11	OS05G0412000					
12	OS01G0210200					
13	OS01G0844900					
14	OS08G0157600					
15	OS12G0586300					
16	OS05G0589400					
17	OS03G0764600					
18	OS10G0562100					
19	OS06G0670300					
20	OS02G0680700					

RESULTS

Comparative mapping showed that MYBAS1 gene is localized on rice chromosome 11 and maize chromosome 4 (Figure 1), MYBAS2 gene is localized on rice chromosome 12 and maize chromosome 1 and 3 (Figure 2), OS01G0176700 gene is localized on rice chromosome 1 and maize chromosome 3 and 8 (Figure 3), OS02G0700300 gene is localized on rice chromosome 2 and maize chromosome 4 and 5 (Figure 4), OS09G0431300 gene is localized on rice chromosome 9 and maize chromosome 2 and 7 (Figure 5), OS02G0685200 gene is localized on rice chromosome 2 and maize chromosome 4 and 5 (Figure 6), OS01G0863300 gene is localized on rice chromosome 1 and maize chromosome 3 and 8 (Figure 7), OS04G0583900 gene is localized on rice chromosome 4 and maize chromosome 2 and 10 (Figure 8), OS04G0600000 gene is localized on rice chromosome 4 and maize chromosome 2 and 10 (Figure 9), OS02G0241200 gene is localized on rice chromosome 2 and maize chromosome 4 and 5 (Figure 10), OS05G0412000 gene is localized on rice chromosome 5 and maize chromosome 6 and 8 (Figure 11), OS01G0210200 gene is localized on rice chromosome 1 and maize chromosome 3 and 8 (Figure 12), OS01G0844900 gene is localized on rice chromosome 1 and maize chromosome 3 and 8 (Figure 13), OS08G0157600 gene is localized on rice chromosome 8 and maize chromosome 4 and 10 (Figure 14), OS12G0586300 gene is localized on rice chromosome 12 and maize chromosome 1 (Figure 15), OS05G0589400 gene is localized on rice chromosome 5 and maize chromosome 6 and 8 (Figure 16), OS03G0764600 gene is localized on rice chromosome 3 and maize chromosome 1 and 5 (Figure 17), OS10G0562100 gene is localized on rice chromosome 10 and maize chromosome 1 (Figure 18), OS06G0670300 gene is localized on rice chromosome 6 and maize chromosome 6 and 9 (Figure 19), and OS02G0680700 gene is localized on rice chromosome 2 and maize chromosome 4 and 5 (Figure 20).

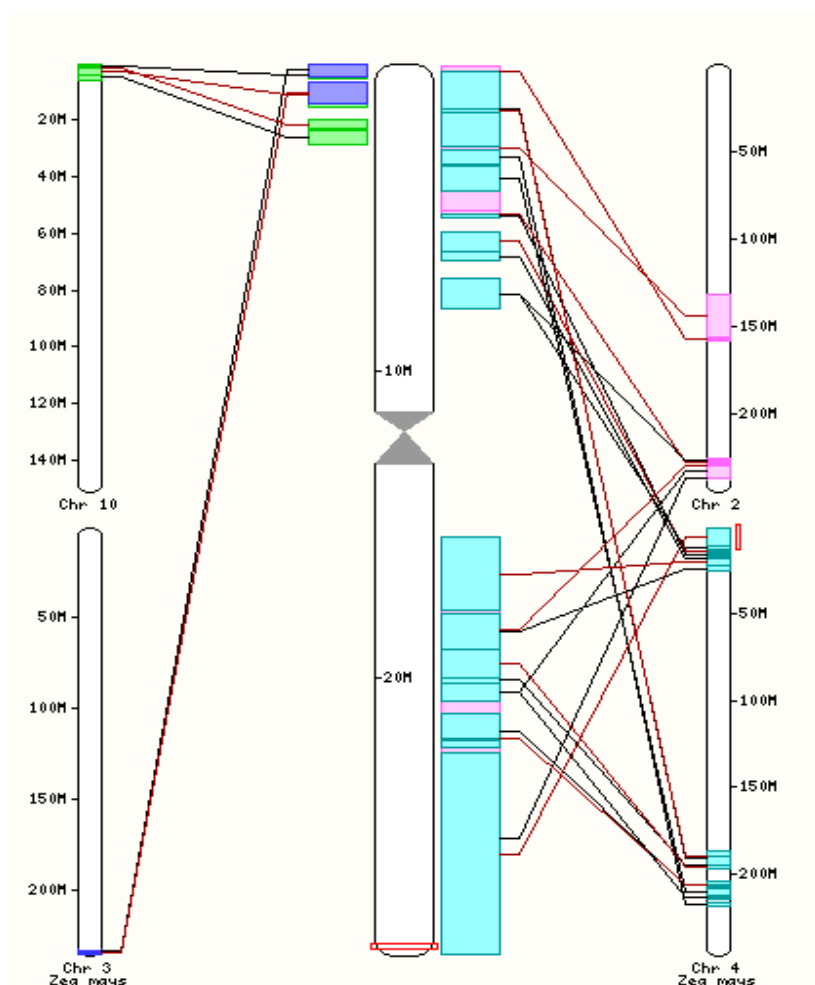


Figure (1): Showing the results of comparative mapping and synteny between the gene MYBAS1 (OS11G0700500) in chromosome 11 in rice (*Oryza sativa Japonica*) and chromosome 4 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

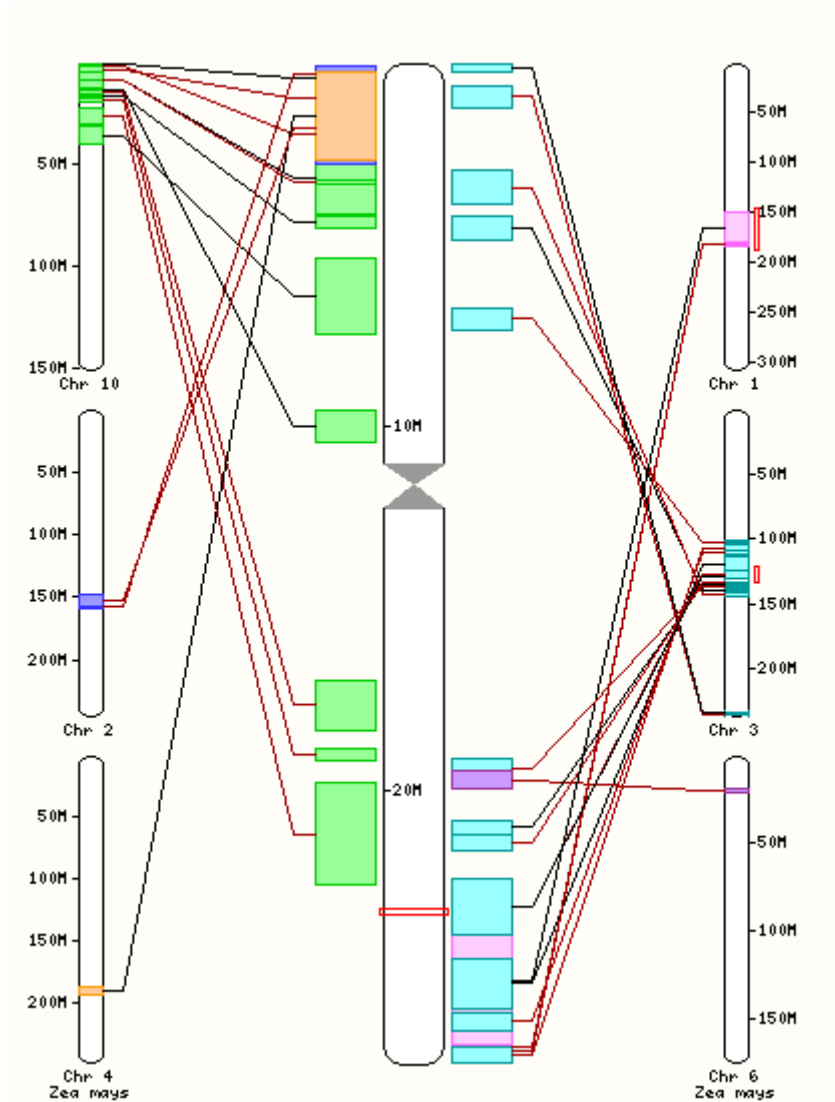


Figure (2): Showing the results of comparative mapping and synteny between the gene MYBAS2 (OS12G0567300) in chromosome 12 in rice (*Oryza sativa Japonica*) and chromosome 1 and 3 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

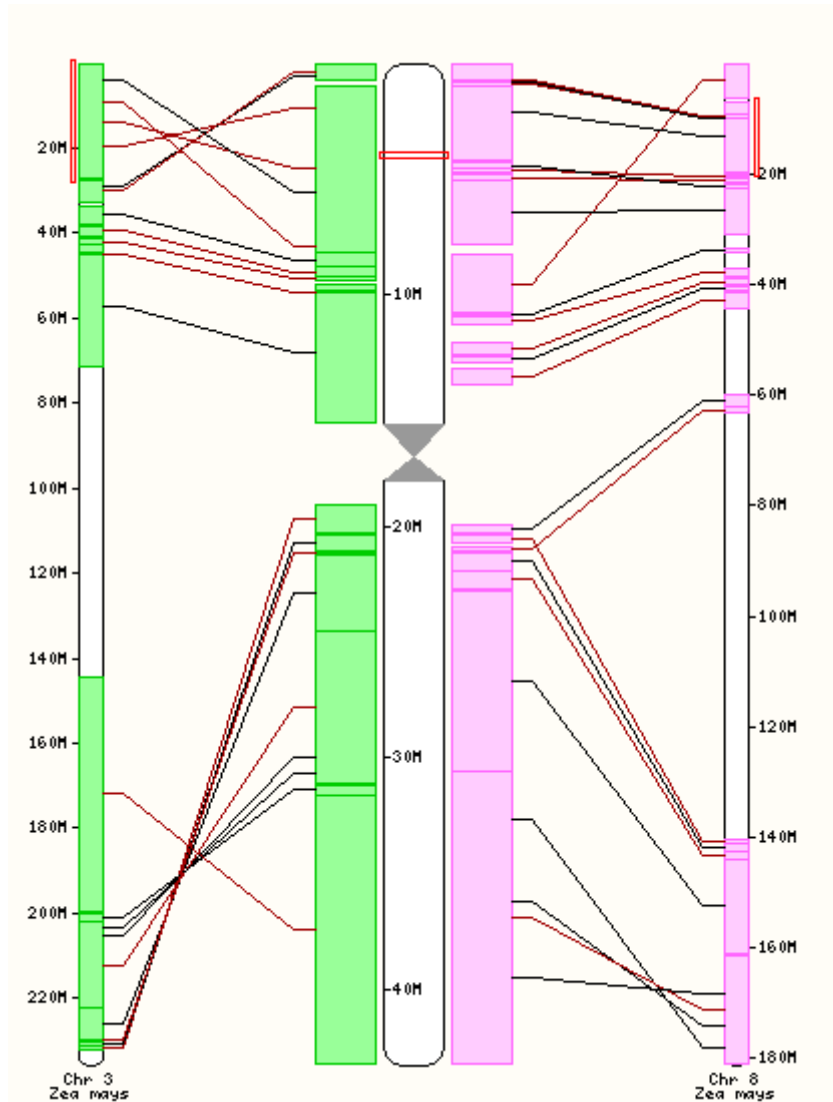


Figure (3): Showing the results of comparative mapping and synteny between the gene OS01G0176700 in chromosome 1 in rice (*Oryza sativa* Japonica) and chromosome 3 and 8 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

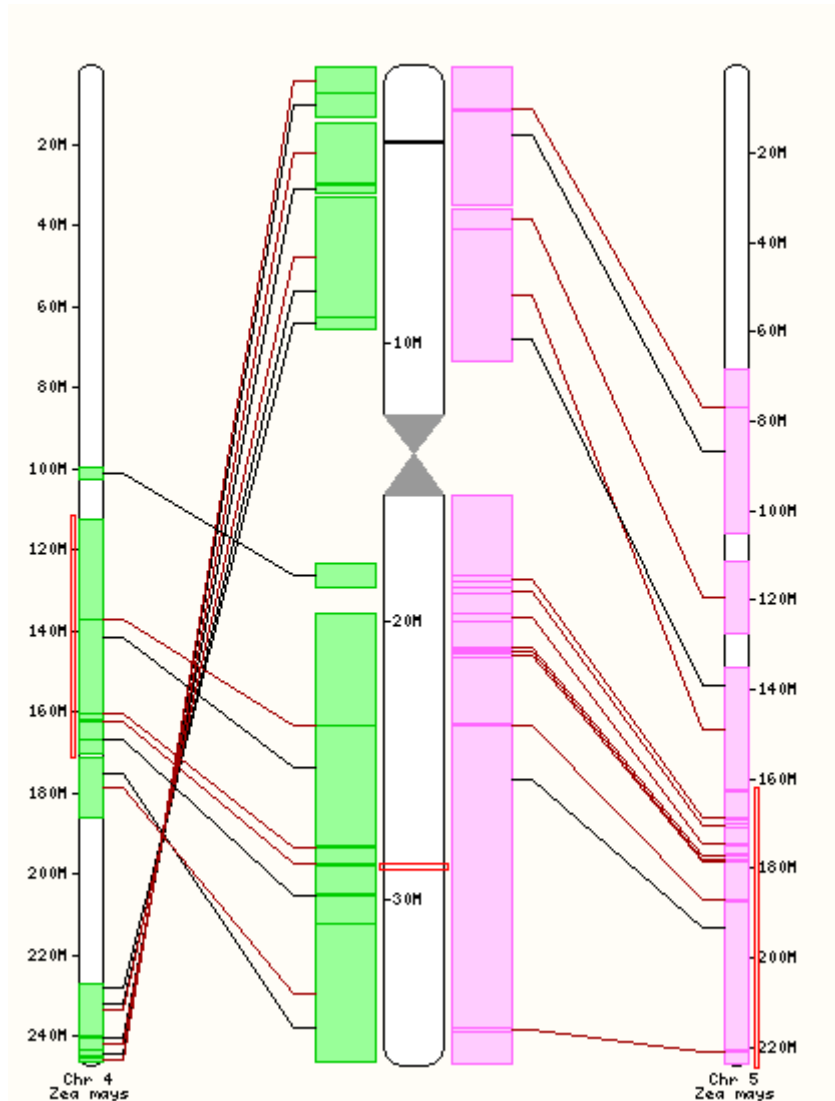


Figure (4): Showing the results of comparative mapping and synteny between the gene OS02G0700300 in chromosome 2 in rice (*Oryza sativa Japonica*) and chromosome 4 and 5 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

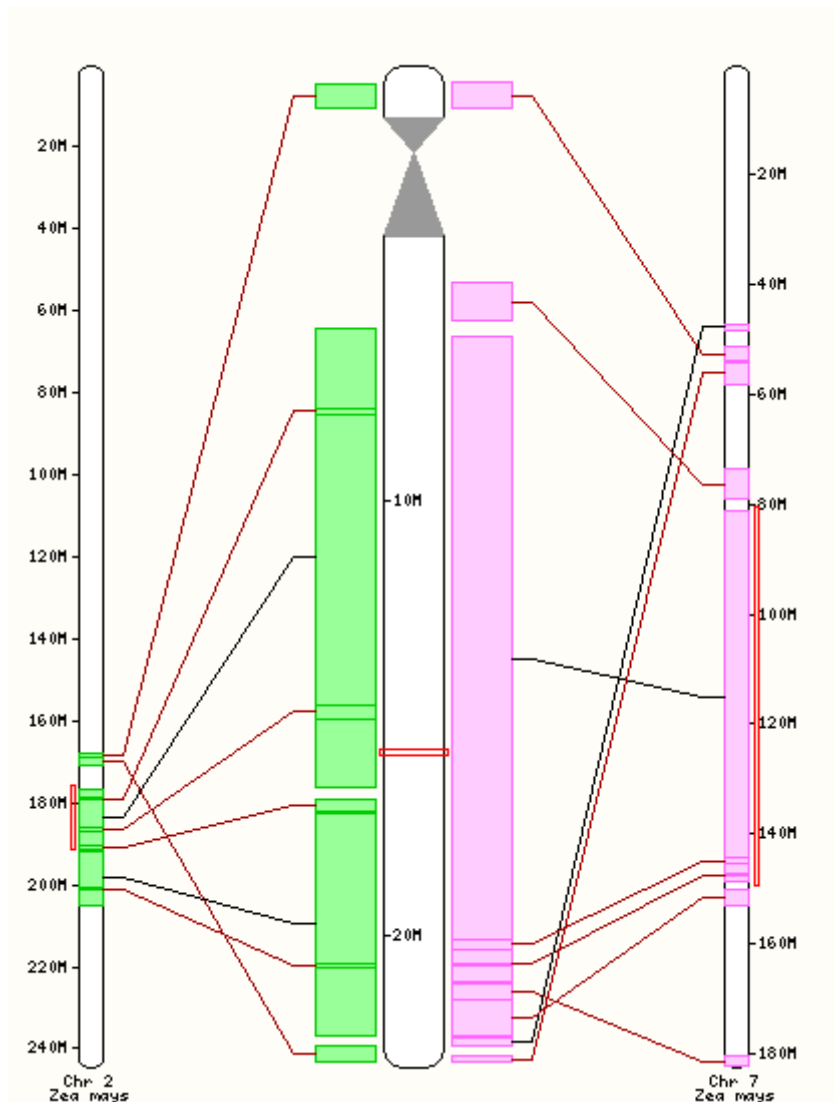


Figure (5): Showing the results of comparative mapping and synteny between the gene OS09G0431300 in chromosome 9 in rice (*Oryza sativa Japonica*) and chromosome 2 and 7 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

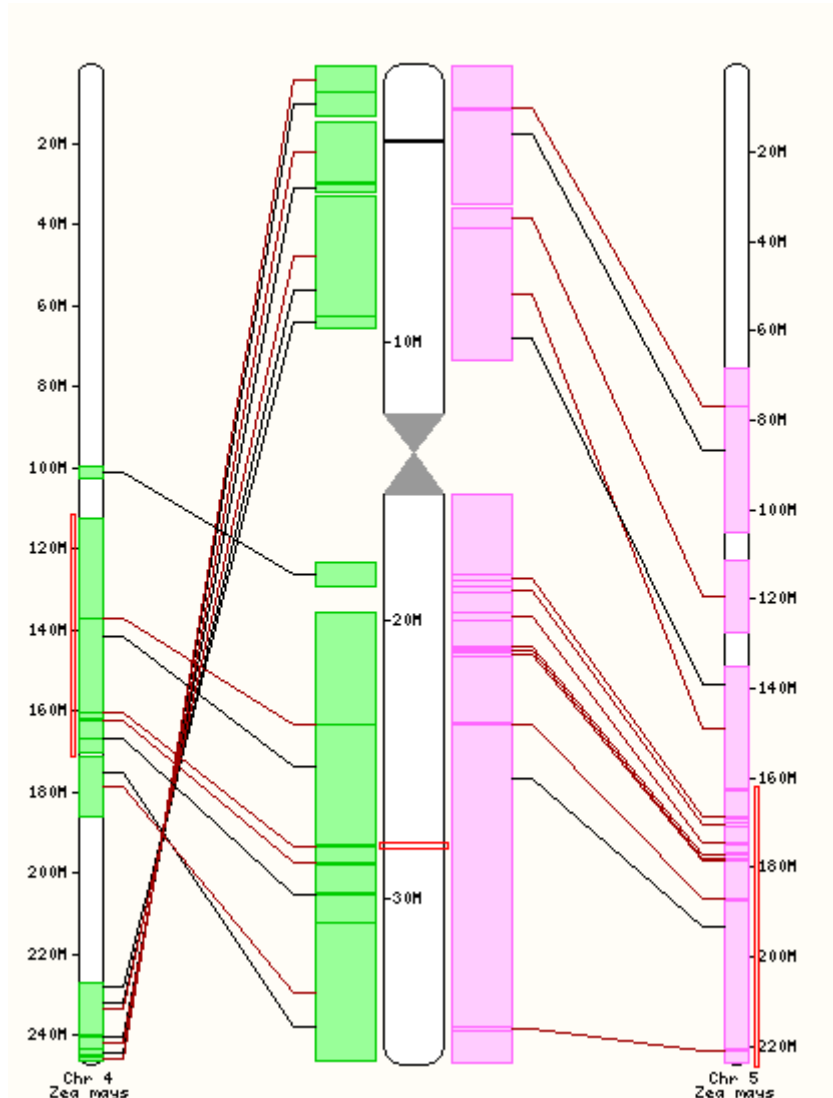


Figure (6): Showing the results of comparative mapping and synteny between the gene OS02G0685200 in chromosome 2 in rice (*Oryza sativa Japonica*) and chromosome 4 and 5 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

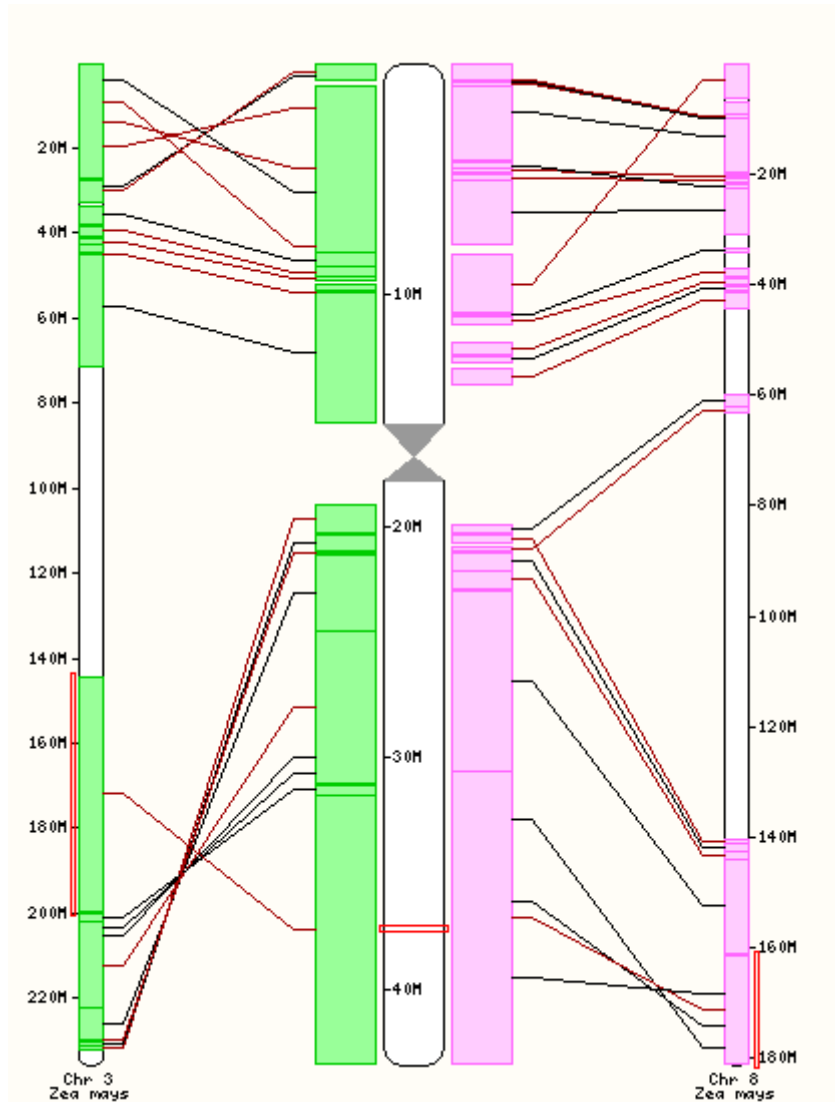


Figure (7): Showing the results of comparative mapping and synteny between the gene OS01G0863300 in chromosome 1 in rice (*Oryza sativa* Japonica) and chromosome 3 and 8 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

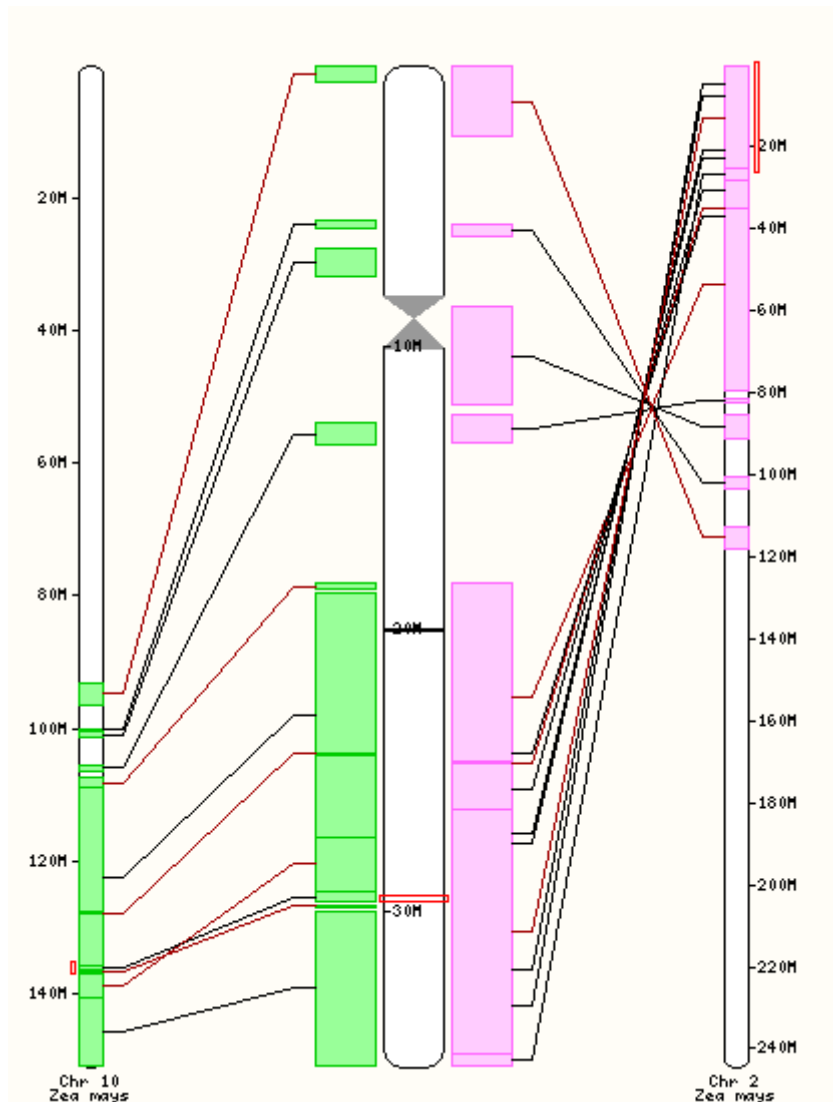


Figure (8): Showing the results of comparative mapping and synteny between the gene OS04G0583900 in chromosome 4 in rice (*Oryza sativa Japonica*) and chromosome 2 and 10 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

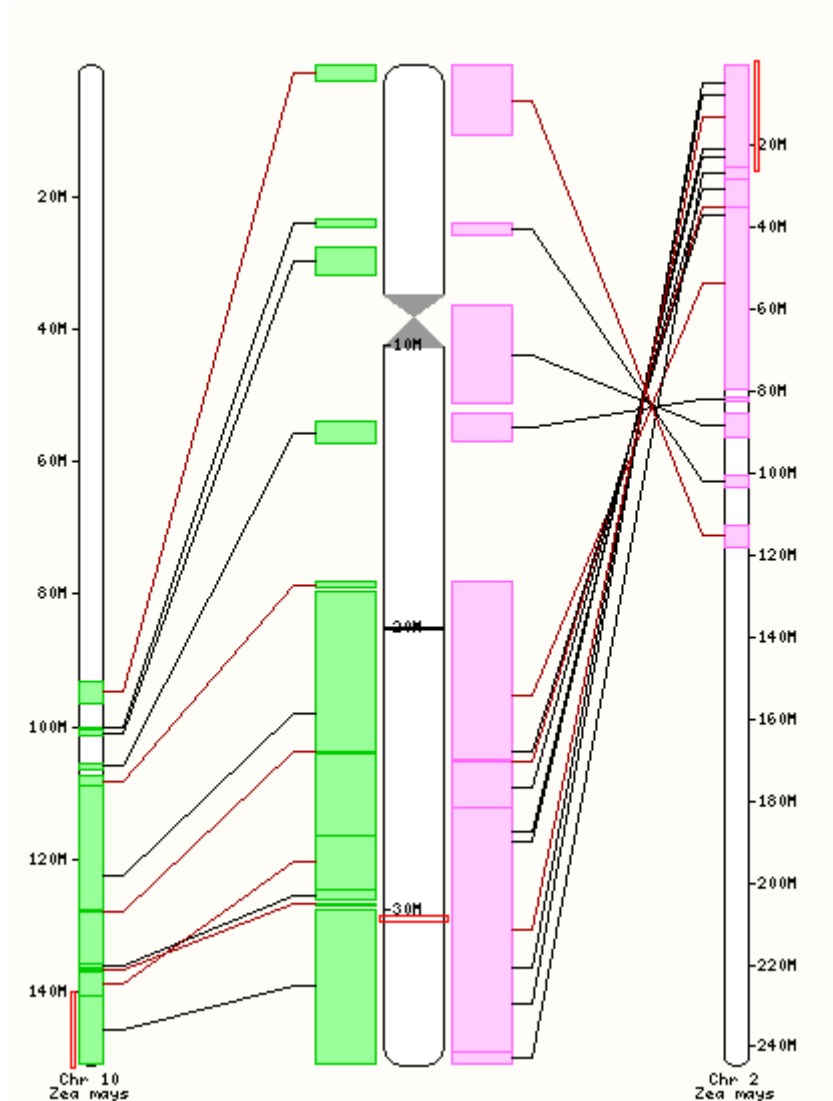


Figure (9): Showing the results of comparative mapping and synteny between the gene OS04G0600000 in chromosome 4 in rice (*Oryza sativa Japonica*) and chromosome 2 and 10 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

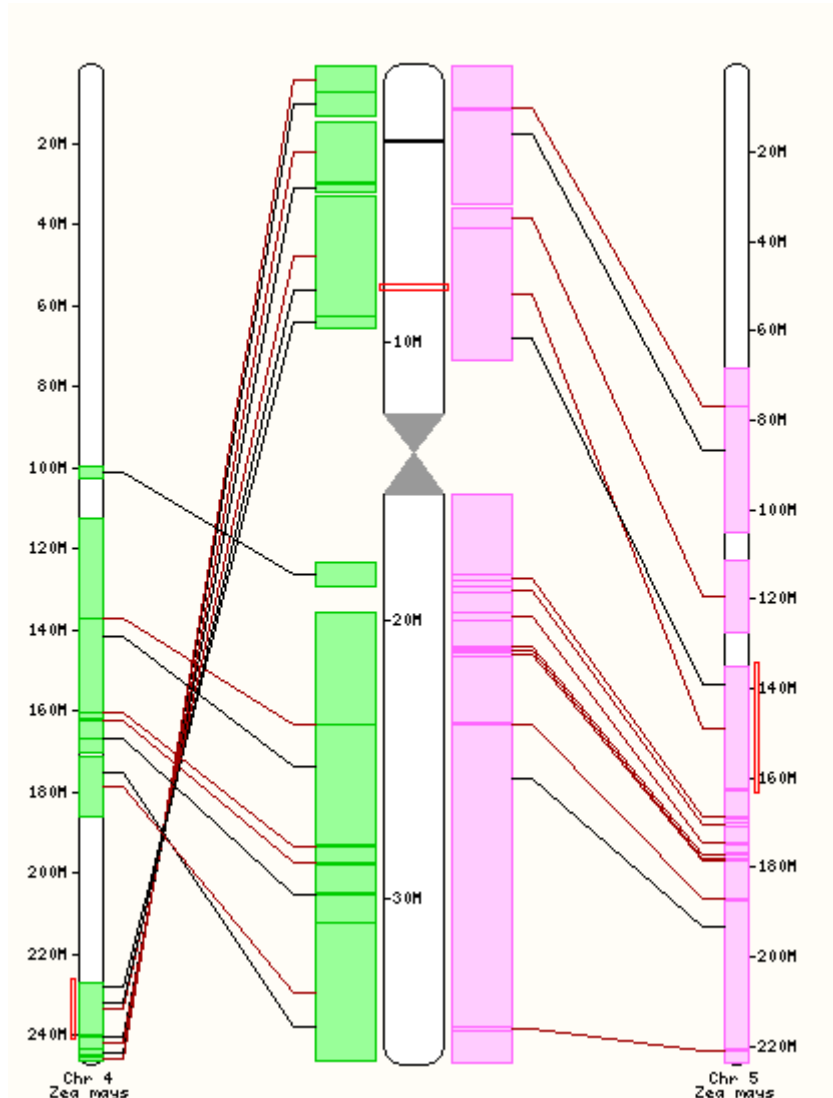


Figure (10): Showing the results of comparative mapping and synteny between the gene OS02G0241200 in chromosome 2 in rice (*Oryza sativa Japonica*) and chromosome 4 and 5 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

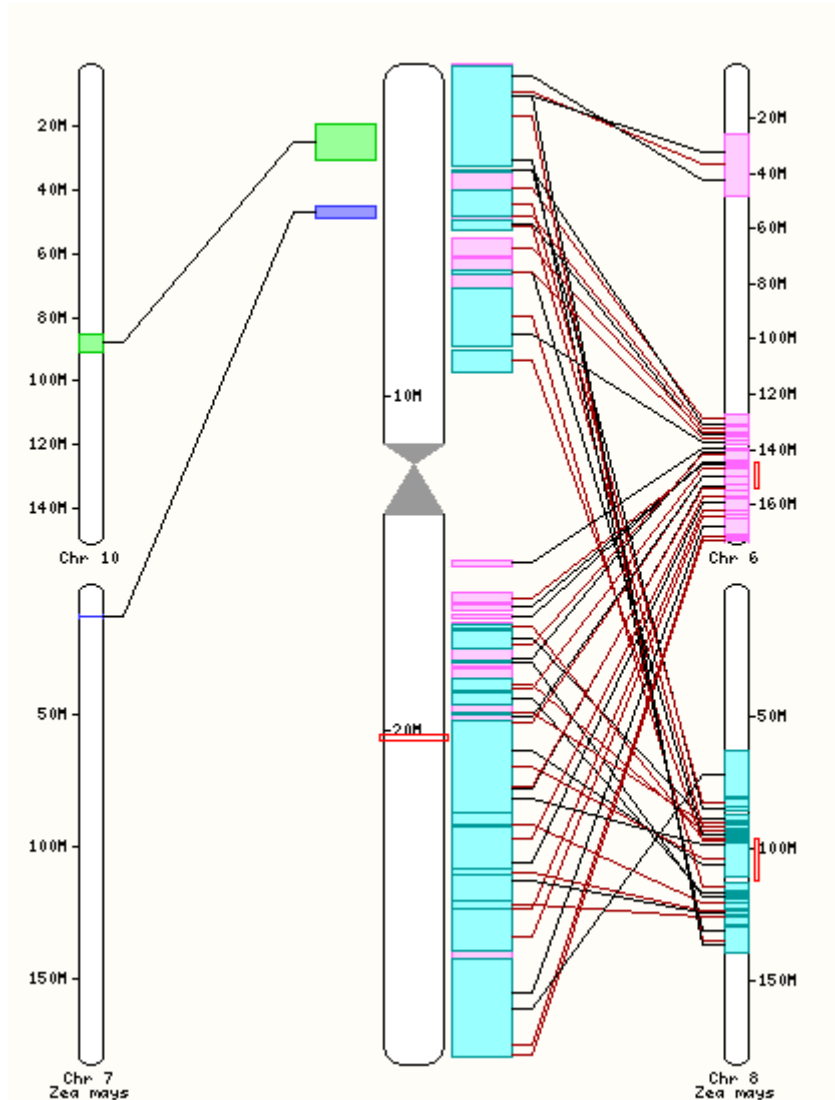


Figure (11): Showing the results of comparative mapping and synteny between the gene OS05G0412000 in chromosome 5 in rice (*Oryza sativa Japonica*) and chromosome 6 and 8 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

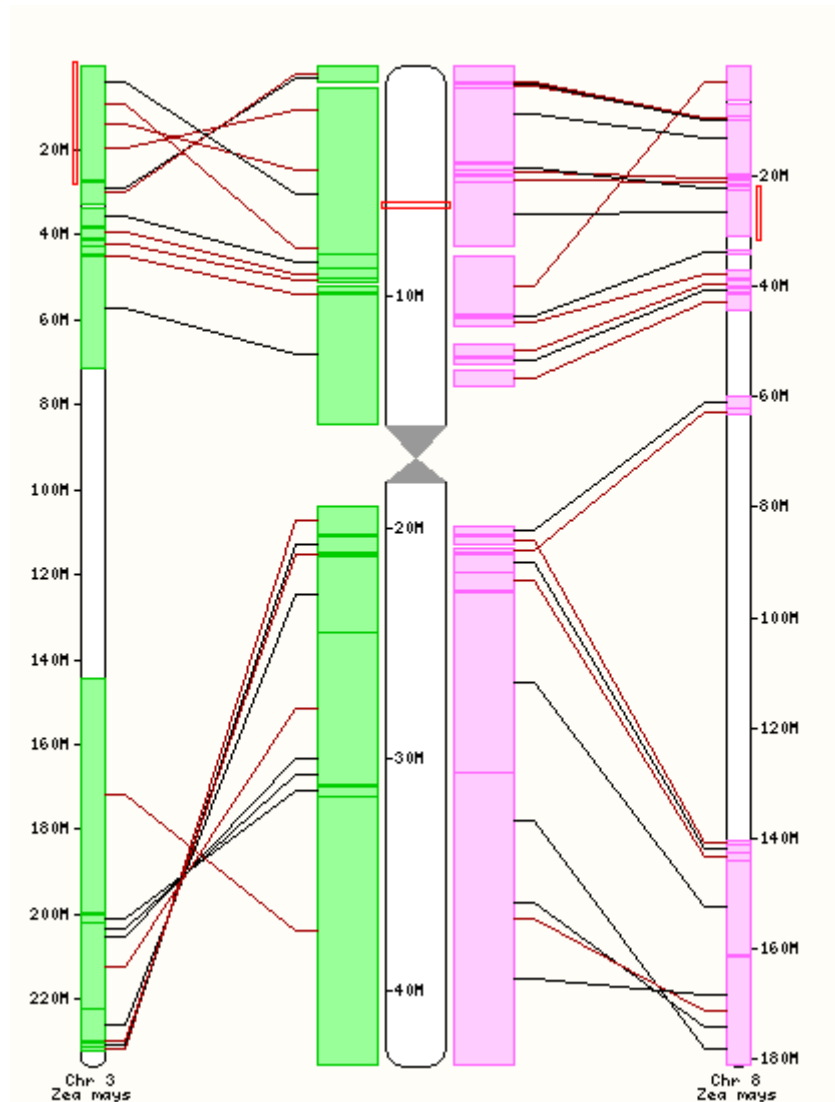


Figure (12): Showing the results of comparative mapping and synteny between the gene OS01G0210200 in chromosome 1 in rice (*Oryza sativa Japonica*) and chromosome 3 and 8 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

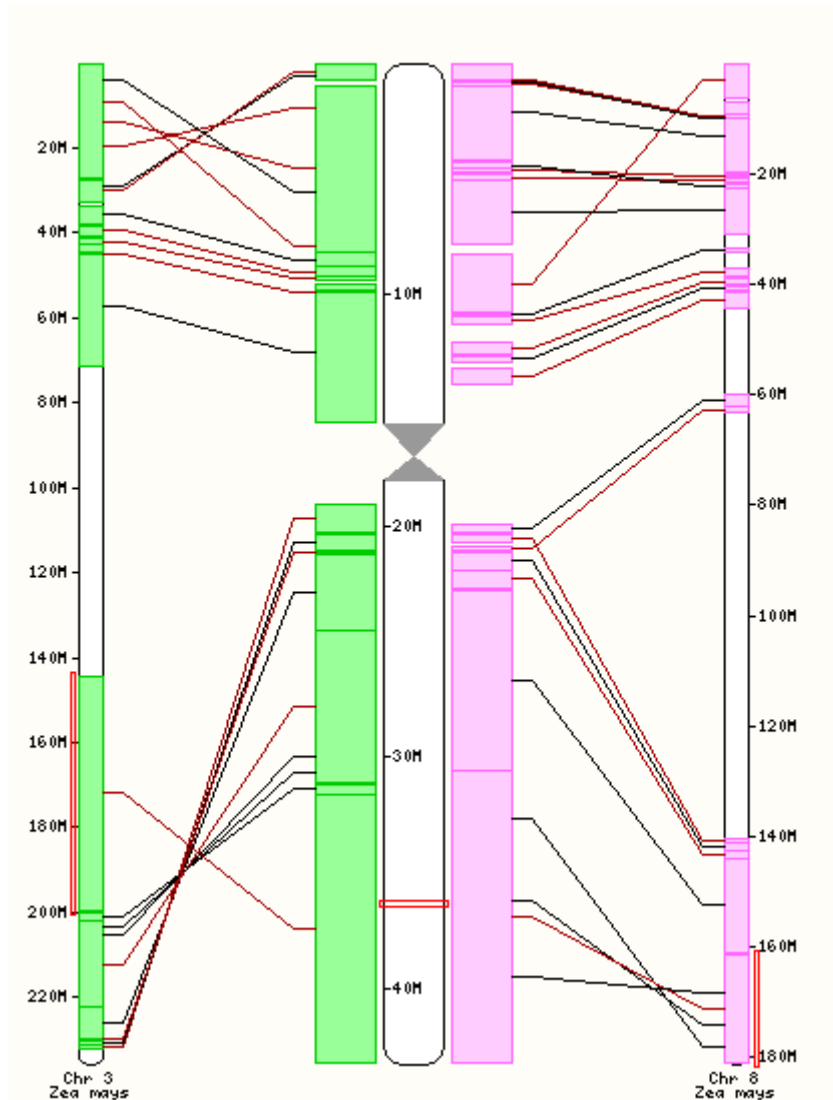


Figure (13): Showing the results of comparative mapping and synteny between the gene OS01G0844900 in chromosome 1 in rice (*Oryza sativa Japonica*) and chromosome 3 and 8 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

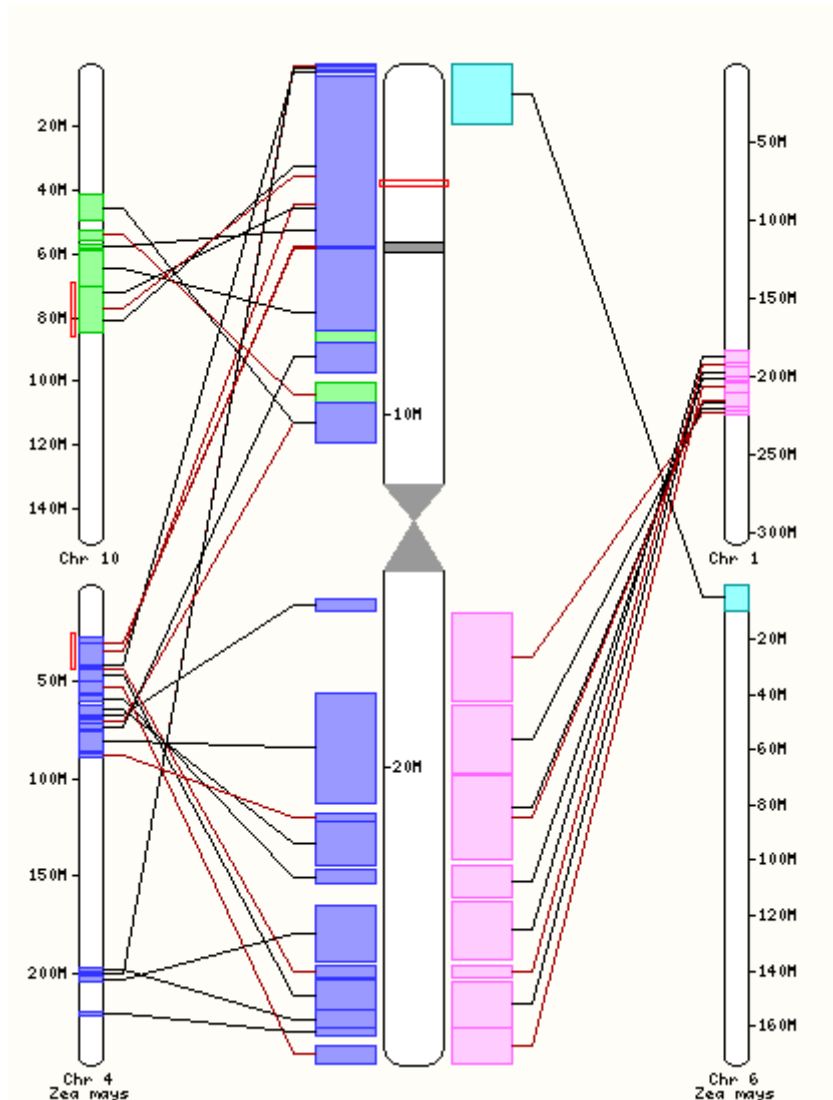


Figure (14): Showing the results of comparative mapping and synteny between the gene OS08G0157600 in chromosome 8 in rice (*Oryza sativa Japonica*) and chromosome 4 and 10 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

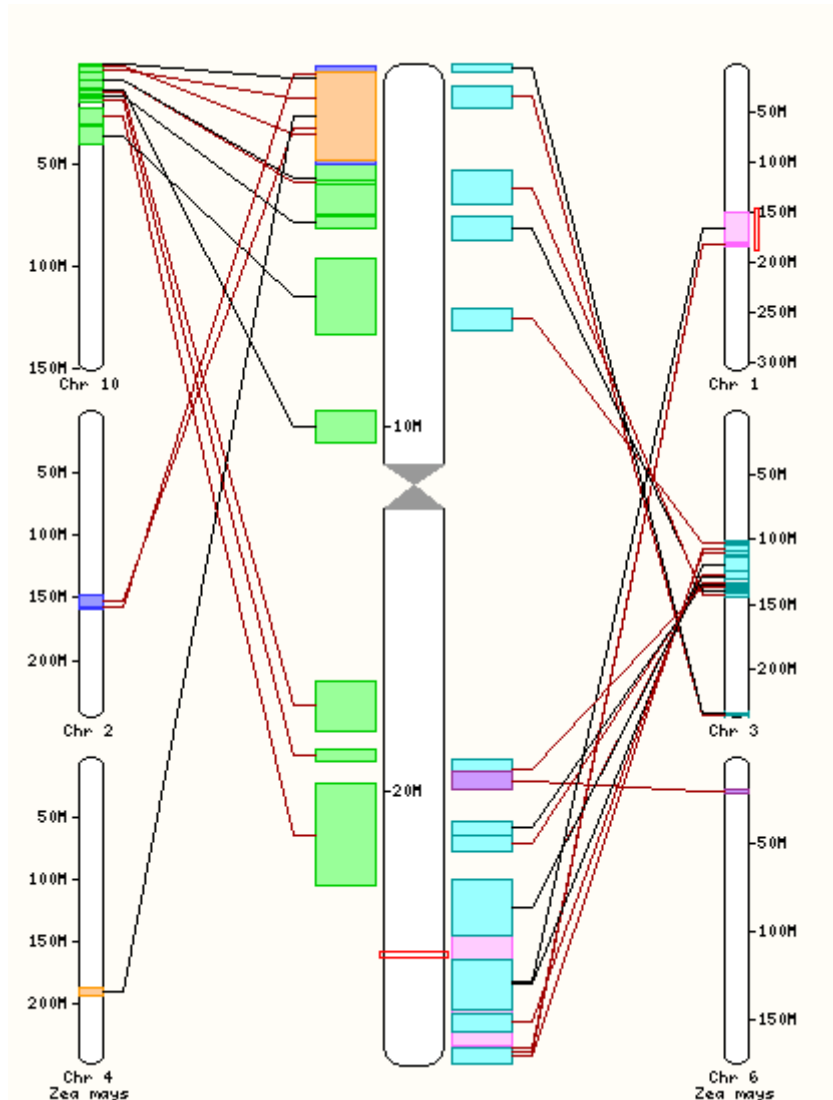


Figure (15): Showing the results of comparative mapping and synteny between the gene OS12G0586300 in chromosome 12 in rice (*Oryza sativa Japonica*) and chromosome 1 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

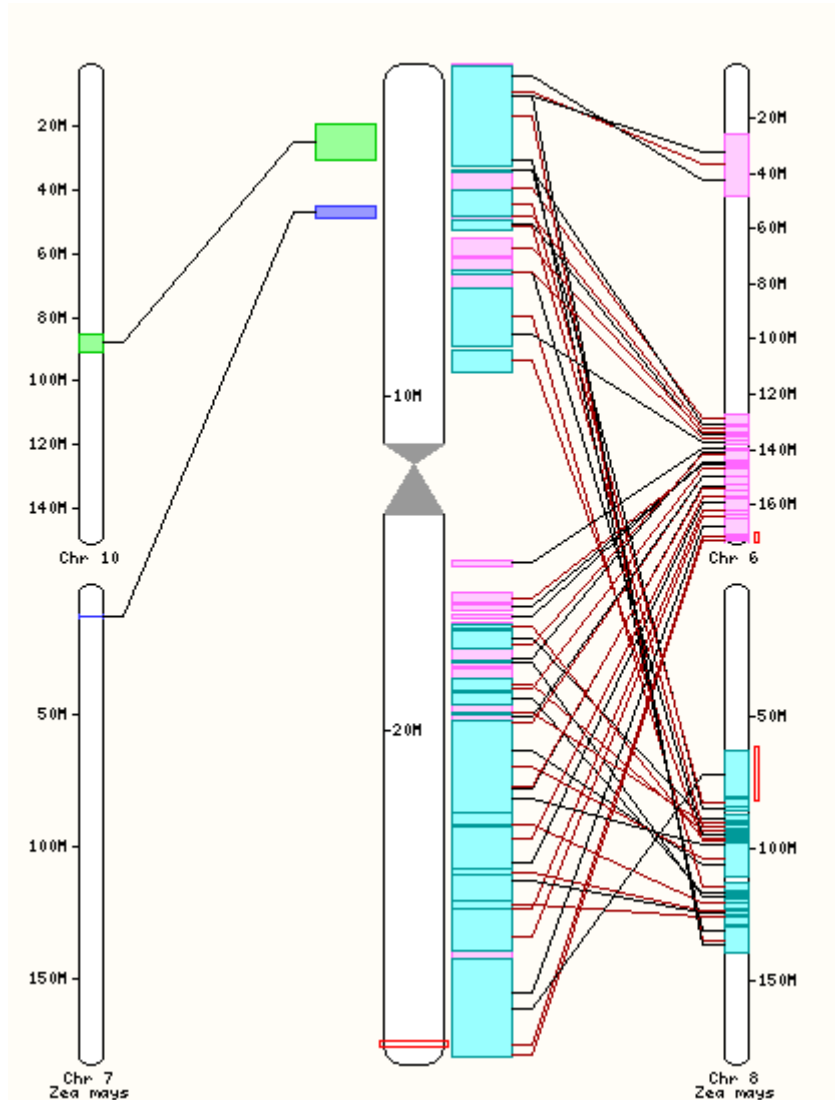


Figure (16): Showing the results of comparative mapping and synteny between the gene OS05G0589400 in chromosome 5 in rice (*Oryza sativa Japonica*) and chromosome 6 and 8 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

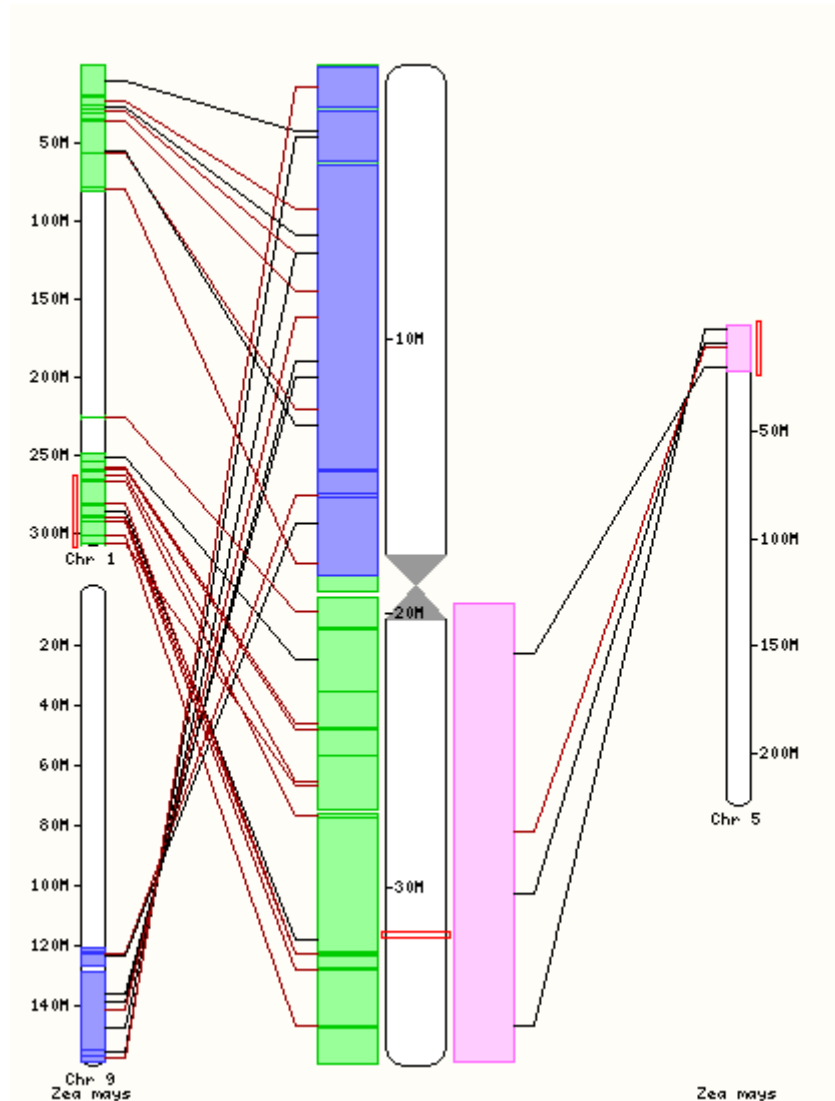


Figure (17): Showing the results of comparative mapping and synteny between the gene OS03G076460 in chromosome 3 in rice (*Oryza sativa Japonica*) and chromosome 1 and 5 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

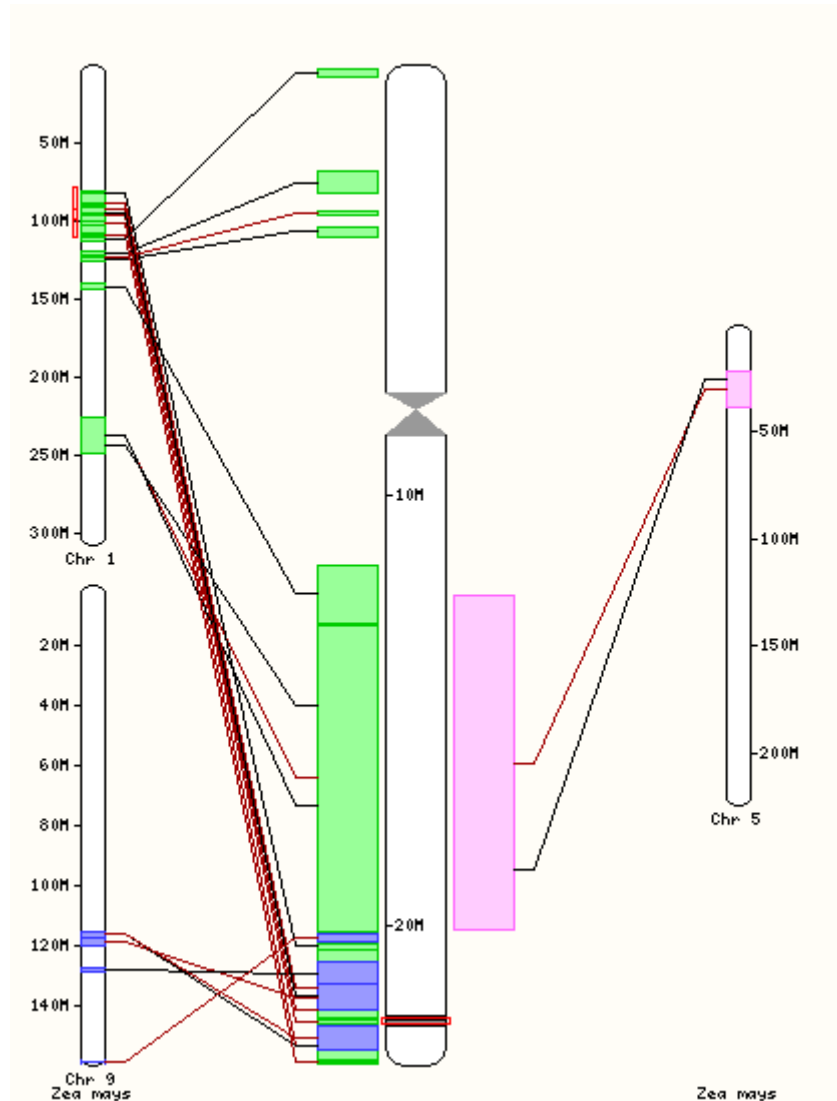


Figure (18): Showing the results of comparative mapping and synteny between the gene OS10G0562100 in chromosome 10 in rice (*Oryza sativa Japonica*) and chromosome 1 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

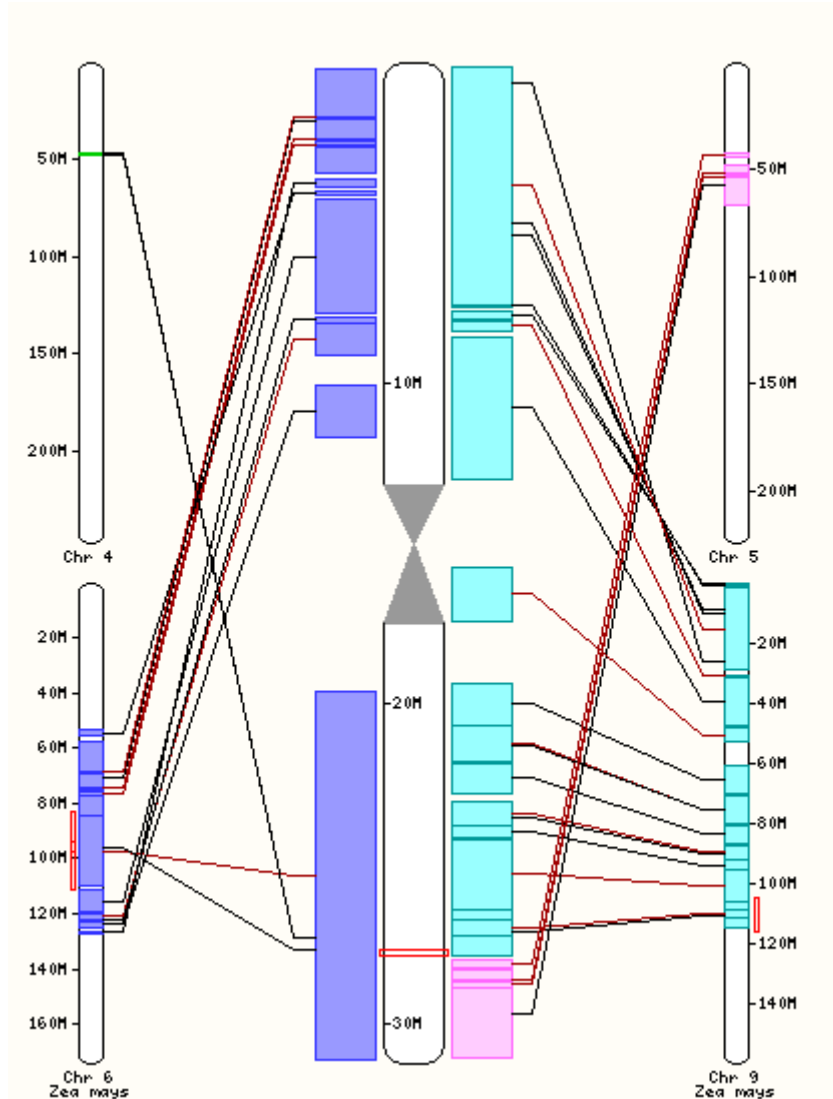


Figure (19): Showing the results of comparative mapping and synteny between the gene OS06G0670300 in chromosome 6 in rice (*Oryza sativa Japonica*) and chromosome 6 and 9 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

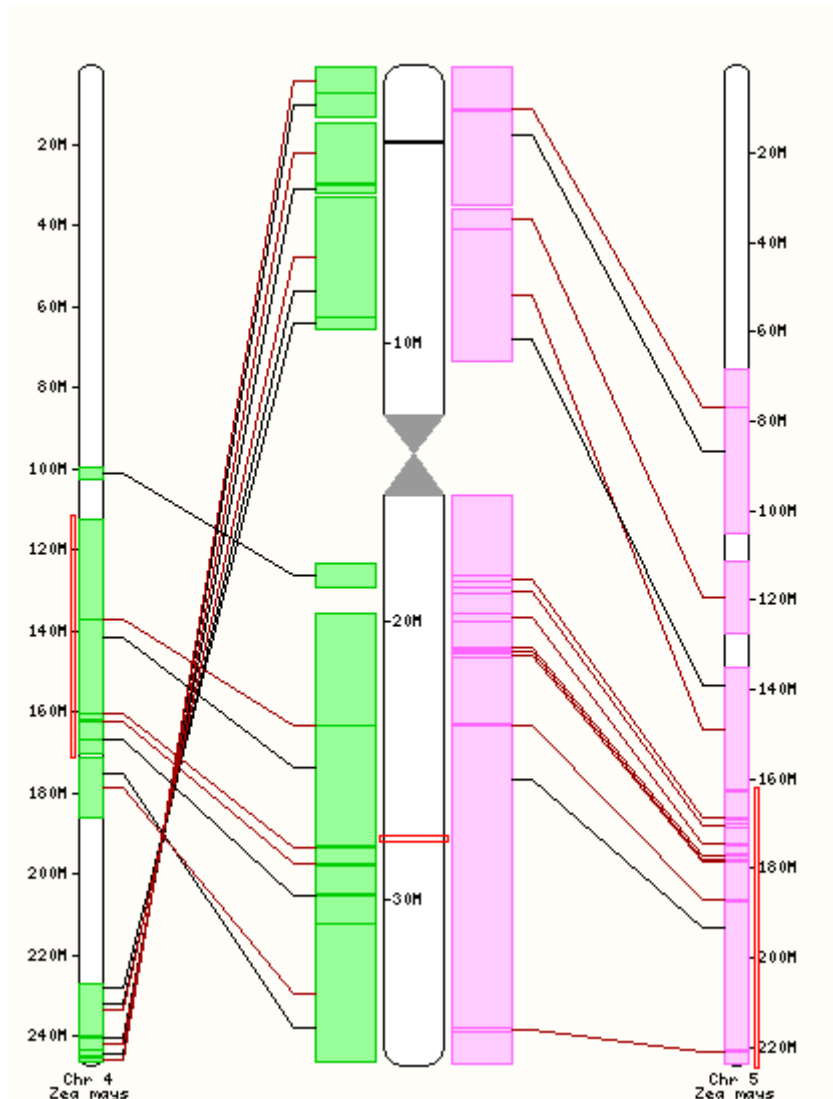


Figure (20): Showing the results of comparative mapping and synteny between the gene OS02G0680700 in chromosome 2 in rice (*Oryza sativa Japonica*) and chromosome 4 and 5 in maize (*Zea mays*). The red boxes indicate the presence of homology in the origin of this gene.

DISCUSSION

Transcription factors are important components of signal transduction networks conveying diverse signals to specific responses. They are being widely used to genetically engineering crop plants, especially in developing plants against drought and salt stress tolerance ⁽²⁷⁾. Alternatively activation of a set of genes by engineering for their transcriptional activation can confer much greater stress tolerance ^(28,29).

Xiong *et al.*, 2014 ⁽³⁰⁾ reported the isolation and characterization of *OsMYB48-1*, a noval MYB-related transcription factor, enhanced drought and salinity tolerance in rice.

In this study, 20 different genes related to MYB TF family were characterized to be used in the strategic improvement of crops for drought and salinity tolerance, and the *in silico* comparative mapping of these genes would open the gate for using them as potential candidate for genetic transformation of crops for drought and salinity tolerance.

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