INTRODUCTION

Wheat is one of the most important and widely-cultivated crops all over the world. The global consumption of wheat, which is close third after rice and maize, (FAOSTAT, 2005), average global wheat production between 1995 and 1999 was 584 million tons per annum but is expected to increase to 860 million tons per annum by 2030 (Maratheè and Gomez-Macpherson, 2000). The demand for wheat is expected to grow faster than any other major agricultural crop and containing high protein content. Wheat *(Triticum aestivum)* considered the main diet for the Egyptian population and it is the main winter cereal crop grown in the country. Improving drought tolerance of wheat has long been a major objective of most breeding programs around the world because water deficits during some part of the growing period are common to most regions of the world where wheat is produced, including Egypt. Wheat breeders are looking for genetic diversity in which drought tolerance and yield potential can be combined.

Wheat covers more of the earth's surface than any other cereal crop, (Curtis, 2002). However, although it takes more land space than other cereals the domestication of it's grains and the development of it's agricultural lifestyles led to significant changes in people's lives, encouraging permanent settlements, development of civilization, and trade. The domesticate wheat produced larger grains and a more productive crop. Wheat in bread form provides more nutrients to Egyptian population than any other single food source .Bread are particularly important as source of

carbohydrates, protein and vitamins B, E (pomeranz 1987), bread prepare with whole grain flours and with multigrain flours (Seibel, 1995, Master,.C.M. and Gould, 1995) .

It is one of the most important crops with annual yields exceeding 500 MT (Figure 1) (FAO, 2004). It forms a stable part of the diet in over 60 countries, being 10-20% of the daily consumption and unique in its ability to make bread So far, the main strategy for improving wheat production has been through conventional breeding method. The successful application of genetic engineering in wheat is dependent on the availability of suitable tissue culture and transformation method (Ye *et al.*, 2002).

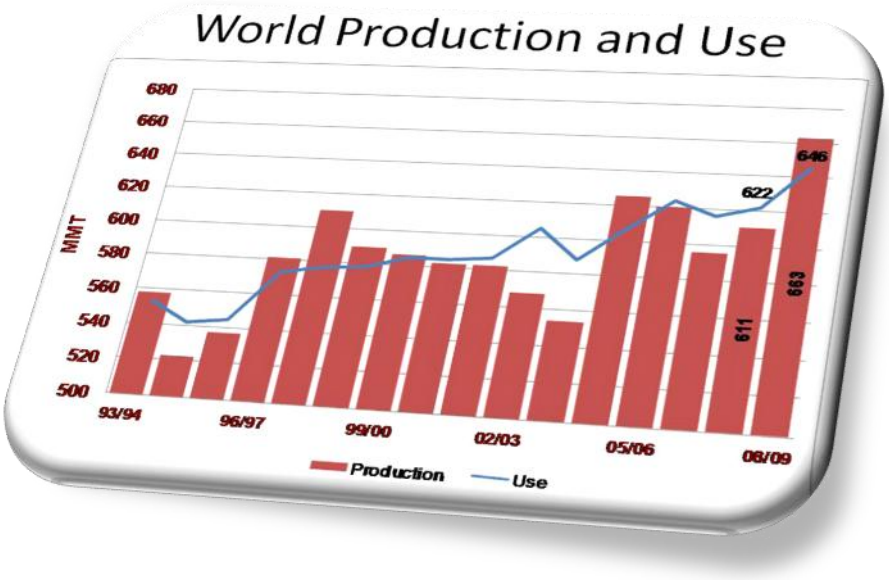

Figure (1): worldwide wheat production and use histograph

Characteristic features of many species of genus *triticum* are the presence of a compound spike with laterally compressed spikelets, each having 2 glumes and a single starch grain. The most important feature is

that all members of this group have chromosomes in multiples of seven, which facilitates the interspecific and intergeneric hybridization. Therefore, the evolution among most of the members of this group is the result of natural hybridization among the wild types. The inflorescence commonly found in the fourteen species of wheat is a spike, containing about 20-30 spikelets each having about four to six florets. One seed is set per floret, although the smaller florets may not bear seeds. Wheat is normally self-pollinating crop. There are four main commercial market classes of bread wheat, namely hard red spring, hard red winter, soft red winter and white wheat. The winter wheat's have a vernalization requirement i.e., they require a cold shock in order to flower. They possess considerable cold tolerance. The spring wheat does not need vernalization and do not have cold tolerance. The red or white colour is due to genes for anthocyanin pigmentation. The hardness or softness of the seed coat refers to the texture of the grain. In this context the soft grain is used for making biscuits rather than bread (Seibel, 1995).

Triticeae is one of the 10 tribes of the subfamily Pooideae, which itself is one of the 5 subfamilies of the family Poaceae (Gramineae). Distributed worldwide, the tribe contains 25 generally recognized genera including those which hold great economic importance like the cultivated cereals. Other tribal members are important as forage and pasture grasses.

Generally wheat can be divided genetically into different tribes. Each tribe has been generated from separate ancestors: Einkorn, Emmer and Spelt (Kent NL. 1994).The most important and relevant crops are four species of the genus Triticum*: T. monococcum (*diploid), *T. turgidum (*tetraploid*), T. aestivum* (hexaploid) and *T.compactum(diploid).*

The most used species for baking purposes are *T. aestivum*, and *T. turgidum. T. turgidum* includes furthermore the durum and dicocconi species, where Italic makes pasta and is also called "Pasta wheat". The features of *T. compactum* are optimal in cake and cookie production (Pomeranz and Williams, 1990).*T. aestivum* is originated from crossing of a tetraploid specie, *T.turgidum* and diploid species, *aegilops tauschii* (Shewry *et al.*, 1999). Bread wheat is a segmental hexaploid (6x), which regularly forms 21 pairs of chromosomes (2n = 42) during meiosis, these Chromosomes are subdivided into 3 closely related (homoeologous) groups of chromosomes, the A, B, and D genomes. Each of these homoeologous groups normally contains 7 pairs of chromosomes (AABBDD) (Sears. 1966) established that each chromosome in hexaploid wheat has a homoeologue in each of the other 2 genomes. This homoeology in hexaploid wheat and also in tetraploid wheat (AABB) allows a range of chromosomal abnormalities (aneuploidy) to survive.

The potential value of cell, tissue and another culture as a tool for crop improvement has been reported over several decades (Green, 1977; Vasil, 1987). The regeneration of whole plant is possible today from cereal species, such as maize (Duncan *et al.*, 1985), rice (Yamada *et al.*, 1986), and barley (Lührs and Lörz, 1988) bread wheat (Redway *et al.*, 1990; Vasil *et al.*, 1990). La Rue (1949) raised the first successful tissue culture in cereals from endosperm. The *invitro* regeneration of wheat is possible from different explants such as mature and immature embryos, grains, endosperm, leaves, shoot bases, and root tips (Sarker and Biswas, 2002). Immature embryo was reported as the best tissue for callus induction and shoot regeneration (Arzani and Mirodjagh, 1999; Sarker and Biswas, 2002); but availability of immature embryo is limited by wheat growing season or requires sophisticated growth chambers. On the other hand, mature grains of wheat are readily available throughout the year, hence can

be used for plant regeneration at any time. High frequency of callus induction is also reported through mature embryo culture in wheat (Ozagen *et al.,* 1998). Establishing an efficient tissue culture technique is difficult in monocotyledonous species particularly in Gramineae family (Sears and Deckard, 1982) compared to dicots. As a member of the family, wheat is also a recalcitrant crop that limits the utilization of tissue culture technique for crop improvement (Vasil and Vasil, 1986). If a suitable protocol for plant regeneration from mature embryogenic callus is available, wheat transformation may be enhanced throughout the year. Although mature embryos yield low callus, their availability throughout the year makes them an excellent ex-plant source.

Recently, a number of advances have been made in the development and optimization of procedures for transformation of several monocot crops including wheat (Altpeter *et al.*, 1996; Blechl and Anderson, 1996; Barro *et al.,* 1997; Cheng *et al.,* 1997; Dobrzanska *et al.,* 1997; Takumi and Shimada, 1997). For practical use in breeding programs, a large number of independently transformed lines are desirable so that those with stable transgene integration and a high level of expression can be selected (Altpeter *et al.,* 1996). The number of transformed plant lines can be increased either by improving the efficiency of transformation or by reducing the time required for their production (Altpeter *et al.,* 1996). Transformation of monocots, however, is still inefficient (Barro *et al.,* 1997; Cheng *et al.*, 1997; Dobrzanska *et al.*, 1997; Takumi and Shimada, 1997). Most procedures, therefore, have focused on shortening the time frame for the production of transgenic plants (Altpeter *et al.,* 1996; Blechl and Anderson, 1996). These procedures typically consist of inducing short-term morphogenic callus from the epidermal or subepidermal cell layer of

the scutellum of immature embryos (Maes *et al.,* 1996). After bombardment, the embryos are kept on callus induction medium with no selection or with low selection (e.g., 1.0-3.0 mg/L bialophos) for a short period (2-4 weeks) for production of embryoids. After this stage, plant regeneration is usually induced on medium containing none or a minimal level of auxin or a mixture of cytokinins and auxins (Altpeter *et al.,* 1996). These manipulations generally produce many regenerate but few true transformants. The transformation efficiencies are usually 0.1-2.5% (Weeks *et al.*, 1993; Nehra *et al.*, 1994; Altpeter *et al.,* 1996; Barro *et al.*, 1997; Dobrzanska *et al.,* 1997). Of the many species of wheat, the hexaploid Bread or Common wheat (*T. aestivum*) is widely cultivated and used for human food, followed by the tetraploid Durum wheat*(T. durum),* and small quantities of the hexaploid Spelt wheat (*T. spelta*) and the tetraploid *(T. polonicum*) (Curtis *et al.,* 2002) Borlaug and his team, using the novel concepts of shuttle breeding and multi-location testing, first developed disease-resistant varieties and then crossed these with the Japanese dwarf variety Norin 10, to produce semi-dwarf, disease-resistant and high-yielding varieties of wheat (Hedden .,2003). This increase must be brought about by improving productivity on land that is already under cultivation and not by bringing new land into use by destruction of forests, grasslands, etc. (Vasil ., 2003) .Genetic transformation, and the production of transgenic varieties with the desired attributes. Production and large-scale cultivation of transgenic crops during the past decade has shown that this novel technology can—when used innovatively and judiciously—address many of the above problems (Vasil. 2003). Tissue culture technology is used for the production of doubled haploids, cryopreservation, propagating new plant varieties, conserving rare and endangered plants, and to produce secondary metabolites and transgenic

plants. The production of high quality planting material of crop plants and fruit trees, propagated from vegetative parts, has created new opportunities in global trading, benefited growers, farmers, and nursery owners, and improved rural employment. (Ye *et al.,* 2002).However, there are still major opportunities to produce and distribute high quality planting material, e.g. crops like banana, date palm, cassava, pineapple, plantain, potato, sugarcane, sweet potato, yams, ornamentals, fruit and forest trees. The main advantage of tissue culture technology lies in the production of high quality and uniform planting material that can be multiplied on a year-round basis under disease-free conditions anywhere irrespective of the season and weather. However, the technology is capital, labor and energy intensive. Although, labor is cheap in many developing countries, the resources of trained personal and equipment are often not readily available. In addition, energy, particularly electricity, and clean water are costly. The energy requirements for tissue culture technology depend on day temperature, day-length and relative humidity, and they have to be controlled during the process of propagation. Individual plant species also differ in their growth requirements. Hence, it is necessary to have low cost options for weaning, hardening of micro propagated plants and finally growing them in the field.

Many publications describe options for reducing costs to establish and operate tissue culture facilities and primarily focus on plant micro propagation. It includes papers on the basics of tissue culture technology, low cost options for the design of laboratories, use of culture media and containers, energy and labor saving, integration and adoption of low cost options, and increasing plant survival after propagation, bioreactors, and outreach of material to the growers and farmers in developing countries.

Bioreactors in plant propagation can produce millions of plants and may cut down the cost of plant production, which is not yet commonly used in developing countries. However, in the near future it could be well integrated into large scale commercial micro propagation in both developed and developing countries. In all cases, such options must be integrated without reducing the efficiency of plant propagation and compromising the plant quality.

Increasing wheat productivity under Egyptian conditions is one of the main targets of wheat agronomists. The yield of wheat is a function of many factors among them the cultivars and nitrogen fertilization being the most important ones. Mosalem (1993) and Khattab (1994) reported that wheat cultivars Giza 164, Sakha 69 and Giza 163 were superior to the rest of the studied genotypes in most major characters which affected grain yield such as flag leaf area, number of spikelets and grains/spike, grain weight/spike and 1000-grain weight. While comparing Sids cultivars with Giza 163, Giza 164 and Sakha 69, Hassanein *et al.* (1997) and El-Karamity (1998) indicated that Sids cultivars surpassed Giza 163, Giza 164 and Sakha 69 in yield components. Grain and straw yields with Sids 8 ranked first, followed by Sids 7 and Sids 6 cultivars, while plant height and number of tillers/plant took reverse trend Sadek (1990) and Salem (1999) indicated that Sids 6 gave the highest grain and protein yield, while Sids 4 gave the highest straw yield. Sharshar *et al.* (2000) reported that, wheat cultivars significantly differed in grain yield and most of measured traits. Moreover, the highest number of spikelets per spike and grain yield/fed was recorded from Sakha 69 wheat cultivar. Nitrogen is the most important plant nutrient needed to obtain high wheat yields in Egypt. Mosalem (1993), Zahran and Mosalem (1993), Darwich (1994), Essay, (1996) and

Zahran *et al*.(1997) reported that plant height, flag leaf area, tillers number and dry weight per unit area of wheat were increased with increasing N level. Several investigators (Darwich, 1994; Essa, 1996; Mostafa *et al.*, 1997; Mosalem *et al*., 1997, Sorour *et al.,* 1998 and Sobh *et al.,* 2000) reported a beneficial effect of nitrogen application on wheat. They reported that numbers of tillers and spikes/m2, plant height, spike length, number of spikelets and grains/spike, grain and straw yields of wheat increased with increasing N level. Therefore, the objectives of this investigation were to study the effect of nitrogen levels on growth, yield and yield components of three wheat cultivars *(T.aestivum L.*) It is a general mention that monocotyledonous plants are more difficult explants for tissue culturing as compared to dicotyledonous because they lack the ability for secondary growth through cambium or cambium like tissues. In current days, the monocotyledonous are cultured and regenerated from different explants materials but plants that recovered are rather smaller and the competence for regeneration was lost within a short period of time. The regeneration of cultured cells or tissue is important through biotechnological approaches, which include genetic transformation of wheat. It can be used convention breeding programs to increase genetic variability and improve some of agronomic traits .Many factors affecting tissue culture response such as: tissue explants, culture medium and its supplement and donor plant growth conditions), Therefore, it is very important to improve wheat by genetic engineering technologies even though it belongs to the plants insensitive to genetic transformation, especially biolistic transformation (Ye *et al.,* 2002). The successful employment of genetic engineering for crop improvement and for basic studies related to gene expression requires a simple and reproducible genetic transformation procedure (Nehra *et al.,* 1994). The ability to deliver and express foreign genes in plant cells are important

steps in genetic engineering of plants. The micro projectile bombardment procedure allowed transformation of cereals at different frequencies depending mainly on the specific parameters of the transformation protocol (Alfonso-Rubi *et al.,* 1999). Yamashita *et al.* (1991) and Hunold *et al.* (1994) independently showed that more than 90% of cells express Gus gene after bombardment with DNA-coated particles in their nucleus. Therefore, they represent the population of cells likely to be transformed if they are able to proceed towards cell division and plant regeneration. Several factors have been described to influence the applicability and efficiency of biolistic gene transfer. Genotypes had been shown to be crucial (Koprek *et al.* 1996; Iser *et al.,* 1999; Rasco- Gaunt *et al.,* 2001 and Ye *et al.,* 2001) stated that wheat transformation efficiency was closely related to donor genotypes. In addition, osmotic treatment of calli mainly applied to reduce tissue damage and consequently enhances transformation frequencies (Vain *et al.,* 1993). Perl *et al.* (1992) recorded that the addition of mannitol improved Gus gene expression. Moreover, Ingram *et al.* (1999) evaluated the effect of osmotic agents (mannitol, sorbitol and a mixture from both) on Gus gene expression and recorded that osmotic treatment containing mannitol combined with sorbitol was more effective. On the other hand, bombardment pressure has a great influence on genetic transformation (Koprek *et al.,* 1996; Rasco-Gaunt *et al.,* 1999). Takumi and Shimada. (1996) produced transgenic wheat through particle bombardment of scutellar tissues, frequency is influenced by culture duration Similar results were reported by (Ozgen *et al.,* 1999).

Detcheva. (1996) designed chromosome analysis intelligent system (CAIS) to perform chromosome analysis and classification. Metaphase and chromosome image processing and analysis are based on analyzing the gray-

level images of biological objects on a microscope slide. The images are composed of biological objects nearly well spread and distinguishable from the background. Image processing methods include preprocessing and image segmentation. Analysis methods extract metaphase and chromosome features to form a symbolic image description. All these methods are applied on large data structures like pixel arrays. A high efficiency and a lower degree of flexibility are required to handle such large amounts of data. To be successfully realized, these methods should be implemented as appropriate efficient procedures. This problem requires special domain-oriented knowledge about image processing and chromosome classification. These studies described the realization of metaphase and chromosome image processing and analysis procedures organized in classes of objects (Bao, *et al.*, 2005; Chauhan *et al.*, 2005).

It is well known that the chromosomes can be investigated by various methods, such as, transmission electron microscope (TEM), high resolution transmission electron microscope (HRTEM), scanning tunnel microscope (STM) and even atomic force microscopy (AFM). These techniques, however, have some advantages and disadvantages, in terms of complexity of the methods and the obtained data. It is well documented that the obtained data from these tools are the first step in predicting and exploiting the nanostructures for novel properties. In spite of the image obtained from these tools is used for determination the size and shape of nanostructures, these images, however, captured and stored a lot of data about the real internal structure of these materials, which has not yet been discovered till now. So, incredible advances in our ability to image and manipulate these structures give birth to the ongoing revolution in nanotechnology.

In the present thesis, the novel visualization Crystal Image Software (CIS) (El-daly and Tolba, 2009) based on combination of image processing; numerical analysis; artificial intelligent and expert system, this technique has been used to image the real internal structure for chromosomes of diploid and hexaploid wheat *(T. aestivum)* which will allow following up the real mechanisms of formation and growth of them from the images. This knowledge will be used in the fabrication of more complex systems for different applications. This technique depends on the fact that the causes of color in many minerals are in response to structural irregularities (Klein and Dutrow, 2008) Such use of this property can be considered as the key factor for mapping the way in which the electron beam of TEM interact with the internal structure of nano material to produce the digital image. This digital image was further used for better characterization of the differences in micro structural field. However, the digital image consists of a square array of image elements or pixels; at each pixel, the image brightness was sensed and assigned with an integer value (from 0 to 255 in the case of gray scale image) that was named as the gray-level.

For better visualization of the image, the gray-level image is transformed into color image and converted into hue, saturation and intensity (HSI) using a miscolored technique. The simplest way of obtaining a pseudo color image from a gray-level image is to use the RGB mode. An RGB color consists of three individual images exposed through Red, Green and Blue filters, which are eventually combined into a single composite color image. Note that, the individual RGB images are not in color. They will still be gray scale images until you combine them into the final color image. This is recognized by many of the popular image

processing programs like Photoshop or Paint Shop Pro; these programs are excellent tools when you want to crop, resize and perform final adjustments to your color images and hence, can offer both more feasible and practical performance at simple tasks and good implementation, which would be impossible by TEM or other tools alone. However, the purpose of RGB color model is to facilitate the specification of colors in some standard, generally accepted way, and is the most common used model in graphics devices. This model however, allows offering color range for the pixels from an integer value 0 to 16777215 (number of colors: 256x256x256). This can be used as an additional parameter for identifying the fine details of the differences in the structure. A standard procedure should always be followed while extracting the nanostructure image to minimize the error from the influence of operational parameters. For this characterization, the image pre-treatments such as, image bright, contract enhancement and image noise removal methods were preliminary processes to obtain better qualification results. More attention should be paid particularly to the resolution and magnification of the image. Also, the technique it is able to detect the structural crystal printing, which means, the process of identification of a crystal structure out of a range of candidate structures that are contained in a comprehensive database (Hanawalt and Rinn, 1936; Mighell and Karen, 1996). Such process of structural crystal printing of nanocrystals cannot be easily achieved till now by the traditional powder X-ray diffraction techniques (López-Pérez *et al.*, 1997; Pinna, 2005) However, the structural crystal printing is extracted from the boundary of the layers that grew from the first stage (nucleus) to the final external form of layer crystal. The boundary in this case is defined as all pixels in the object having an edge neighbour in the background. The technique is also able to detect the arrangement and configuration of any Pico-structure of

the crystal phases which will explain the mode of formation of the nanostructure. Crystal phases are referred to in this Thesis simply as regions in space with homogenous physical and chemical properties. The arrangement of colors which appeared in all images is related to the change in the rate of crystal growth during synthesis process and the crystal phases.