

PAKISTAN REQUIRES MODERN TECHNIQUES AND PROPER MANAGEMENT TO BOOST UP ITS AGRICULTURE AS COMPARED TO CHINA

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ABSTRACT

In-addition to classical breeding, high throughput techniques for DNA and RNA quantification are also the demand of time in order to boost up agriculture in places like Pakistan. In the modern world, usage of high throughput techniques is very common to flourish agriculture sector. As in China, during the past five decades rice production has been increased three times more; credit goes to the increased grain yield but not to the planted area. In fact, the agriculture sector cannot be drive properly in any state unless the solutions are found for elementary issues of management. Here, in this review article, we have not tried to co-relate the performance of agriculture sector between two countries only but explained briefly the basic hurdles of management that are destroying the crop yield and their management in the agriculture sector of Pakistan as compared to China. On the other hand, with respect to current advances in the plant science, as the sequences of many plant genomes have been released and a huge array of molecular markers exists for crops. Meanwhile, the power of genomics for practical breeding is one of the best tools of recent years. We suggest that a sustainable increase in crop production is attainable in Pakistan through the adaptation of these modern techniques including; genome-wide association (GWA) studies and emerging RNA sequencing (RNA-Seq) for the basic research.

Keywords: Agriculture, High through-put techniques, Management, China, Pakistan

INTRODUCTION

The importance of agriculture cannot be avoided in any state, while the demand for agriculture is increasing dynamically in the developing world day by day. If we take Asia, 90% of the world's rice is growing by it. As compared to population, this rice production is not enough in Asia where about two-third of the world people are living (Khush and Virk, 2000). Now, it is a big challenge for the global agricultural system to increase the agricultural productivity from the existing land area (Robertson and Swinton, 2005). So, in order to boost up agriculture there is a need to control the various bottlenecks in its pathway like biotic and abiotic stresses on one side while poor management practices on another side. Huge yield losses occur every year as a result of these stresses. According to Food and Agricultural Organization (FAO), world's total area (12.78 billion ha) is affected by salt in soils. It is a seriously affecting problem that one third of all irrigated land in the world is exaggerated by salt (Mass and Hoffman, 1977). This results significantly in the reduction

of plant growth and development (Ashrafuzzaman *et al.*, 2002). According to Chinese researchers, the role of crop management is better than growing more area and new varieties for yield (Fang *et al.*, 2004). Their mean is to manage crop in such a manner that future crop production is not threatened. In places like Pakistan, the situation is not different. A huge part of agriculture production destroyed in the country due to lack of proper crop management practices in various fields like unavailability of pure and proper seed, crisis of energy and shortage of water, unavailability of required fertilizer, feeble input and output markets, the stumpy level of education of poor farmers, poorly functioning extension services etc.

The concerned review article deal with various top most hurdles, their management and the importance of most accurate high throughput techniques for agriculture boost up in Pakistan by keeping the development of China in agriculture as a model. However, a great amount of information and diverse technology platforms of it will pose multiple opportunities for plant breeding community, professional scientists and related students particularly.

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The importance and a co-comparison of Agriculture between two countries

Agriculture is a vital industry in China and Pakistan. China is a worldwide major producer of rice, cotton, wheat, potato, sorghum, peanut, tea, millet, barley, oilseed, pork, and fish. Pakistan also is one of the world's leading producers and suppliers of agricultural products (FAO, FAOSTAT). The soil of Pakistan is full of all basic nutrients and has capacity to yield more and more but the constancy of Pakistan's agricultural sector is uncertain due to many reasons. Since the early 1960s significant development and output in agriculture has been achieved from the country but unfortunately, the country could not be benefited fully from the fertile soil and one the best irrigation system of the world (Indus River irrigation system). Not only this, if we see the record of agricultural growth in Pakistan, it decreased constantly during the sixty five years history of the country (Fig.1)

Both neighbor countries (China and Pakistan) have comparable land holdings and agricultural practices, with the exception of research and technology. In China, new technologies of crop management have been developed by using systems approaches, while in Pakistan there is nothing else. The Government policies also play a central role in the growth of agriculture sector. As in China the government is supporting fully to develop the high-yielding varieties through semi-dwarf, hybrid, and new plant types by using an inter sub-specific heterosis and a combination of ideotype approach. As a result of this struggle, the hybrid (Liangyoupeijiu and Xieyou9308) varieties of China have yielded 8-15% more than hybrid check varieties. As a result of this policy during 1998–2005, about 34 “super hybrid” rice cultivars were sown in China that resulted an additional yield of 6.7 million tons (Chang *et al.*, 2007). Similarly, if we compare two countries on the behalf of Control of major threats in agriculture we see that China is so far from Pakistan; as in 2005 when brown plant hopper outbreak in China and caused a destruction of 2.77 million tons of rice but the Chinese scientists have already isolated and cloned many genes against that disease from various resources (Zhang, 2007), while in Pakistan cotton leaf curl virus (CLCV) is until a major problem from last two decades. Not only there is the presence of these threats in agriculture of Pakistan but the genetic background of crops is also narrow, there are

weak extension services, un-protection of irrigation system, and oversimplified crop management. Despite all these challenges, it is also possible to increase crop production in Pakistan through the application of current latest crop production practices along with incorporating modern agricultural biotechnology (McMichael, 2001). Now, in the country there is a need to take abrupt actions to preserve the natural resources in the form of soil and water that should be socially adaptable and environmentally acceptable (Galvani, 2007; Pitman and Lauchli, 2002).

Major bottlenecks affecting crop production in Pakistan

First of all we want to explain the major factors that are affecting agriculture in Pakistan. The Agricultural sector play a central role in Pakistan's economy and 32% of its gross domestic products belong to this sector. In spite of it, Pakistani agriculture is not producing the actual potential due to various major threats. Such as Pakistan cotton (major crop) production for 2009/2010 was forecasted at 10.5 million bales, with a possible expected shortfall of 12% or more than one million bales (Syed, 2009) due to many factors. Let us try to explain these factors step by step.

Global climate change and floods in Pakistan

The global climate is changing rapidly due to various natural disasters such as drought, rainfall and floods (Van Aalst, 2006). This increase in temperature not only increases grain sterility but a big proportion of grain remains empty that directly reduce the yield (Matsui *et al.*, 2000; Yoshida *et al.*, 1981). However, in Pakistan for the last few decades, drastic changes in climatic conditions have affected the cultivation and quality of various crop varieties (Naz *et al.*, 2006). It is estimated that about 1.3 million hectares of some important seasonal crops like sugarcane, maize and rice destroyed due to heavy monsoon rains that cause the floods in late July. Under these circumstances repeated use of selected crop breeding lines in various breeding programs not only limits the genetic basis but also develop susceptibility to various abiotic and biotic stresses (Galani *et al.*, 2010).

Water-logging, Salinity and drought

Salinity and drought are another serious problems that are not only affecting one third of all irrigated land in the world but are reducing the growth and agricultural productivity more than any other factors (Karakas *et al.*, 1997). The situation is not different in Pakistan; the salt is also affecting 2.8 million hectares of irrigated land here. Saline and saline sodic soils have affected major agronomic traits than any other factors in Pakistan (Ashrafuzzaman *et al.*, 2002). While, the measures taken so far are quite inadequate for such issues. These factors not only affect the agriculture sector but the social fabric of Pakistani society. In 1999, it was estimated that a cost of about 14 billion Rupees is lost annually by Pakistani farmers due to water logging and salinity (Haider *et al.*, 1999).

Biotic stresses (CLCV, mealy bug and others)

Biotic stresses like leaf blight and bacterial blight, brown plant hopper and stem borer, root smut and false smut, bowl warms and mealy bug etc. are also destroying crops in Pakistan, among crops, cotton shares most in Pakistan's economy through the contribution of 8.5% in GDP (Arif *et al.*, 2012), not only this it is fulfilling 64% demand of edible oil and also a source of 64% foreign exchange (Arshad *et al.*, 2011), but due to many sucking and chewing insects about 20-40% loss occur annually in cotton production (Ahmad, 1999; Saeed *et al.*, 2007). Similarly, Cotton Mealybug (*P. solenopsis* Tinsley) a sucking insect is also destroying the whole crop and cannot be avoided in Punjab. It is also reported that in 2005, a new cotton pest "*Phenacoccus solenopsis*" appeared in Punjab and Sindh and subsequently spread to weeds, ornamental plants and vegetables (Arif *et al.*, 2009; Abbas *et al.*, 2010a) and cause a big destruction.

Lack of Education, Research and Extension Services

Technological change and technical efficiency along with healthy agricultural extension services may cause a revolution in agriculture anywhere in the world. According to record, a leading growth in agriculture production take place in history of Pakistan but the estimated difference is so wide (Ali, 2000; Kemal, 2002). However, various researchers have point out that agricultural extension in Pakistan has so many challenges such as hierarchic top-down

system of administration, weak research-extension education linkages (Khan, 2006), poorly conducted meetings and field demonstrations (Malik *et al.*, 1991), lack of communication skills and training of extension field staff (EFS), non-use of extension methods effectively (Choudhry *et al.*, 1993), lack of training facilities for EFS (Khan, 1991), lack of fundamental facilities like transportation, accommodation, special incentives and technical knowledge for EFS to perform their job (Zehri, 1993).

Crop management can play a role in agricultural development of Pakistan

Yes, crop management practices can play a central role in agricultural development; not only in Pakistan but in the whole world. Despite these challenges, good research strategies and actual management in crop can drive increase crop production in Pakistan. The situation is so worse in Pakistan. More specifically, increased efforts are needed to raise crop productivity from salt and drought affected land by combining well crop production and high management practices (Galvani, 2007; Pitman and Lauchli, 2002). It is also claimed by one study that more than 50% loss in the yield of major crops take place due to biotic and abiotic (Wang *et al.*, 2009) and in future such condition may also be expected (Lane and Jarvis, 2007). Improper control of weeds is also losing 17-25% of crop annually (Shad, 1987). Similarly, on monetary basis loss to wheat crop in Pakistan due to weeds is an amount of Rs. 28 billion, while only in Khyber Pakhtunkhwa (formerly NWFP) it amount to Rs. 2 (Hassan *et al.*, 2008). These figures deserve an efficient interest to crop management in the country.

Now, there is a need of an efficient pre and post-harvest system in Pakistan aims to diminish losses and continue the quality of the crop until it reaches the final consumer. Both food security and income increase automatically when food losses are minimized. Therefore, Crop management strategies can solve the problem of agriculture in Pakistan at basic level for achieving high resource-use efficiency through the full expression of yield potential. Government policy related to domestic market liberalization and crops self-sufficient policy for productivity investment can play a central role in agricultural development. Chinese governments as well as Chinese crop

researchers believe in planting high yield varieties than planting new area (Fang et al., 2004), for this purpose new technologies for crop management have been developed in China in the past. While, the Scientists in the Jiangsu province that is very close in resemble to the Punjab province in Pakistan have developed new ways of crop management and various useful morphological parameters at different growth stages of plant for better yield of grains (Su et al., 2002).

Now, Pakistani researchers also need to modify their crop varieties with high yielding, salt and drought resistance, having high shelf life and with better nutritional values in order to meet and compete the challenges of 21st century. They also need special training while, the universities of agriculture also need to change their syllabus according to new agricultural requirement of the country. Overall there is a need of replacement of field crop with horticulture crop for export, strong horticulture organization, training of people in horticulture science, similarly, precession land leveling of soil to save water can immediate boost up agriculture in Pakistan. Meanwhile, Inputs from agronomists and physiologists is also vital for success in this endeavor.

The Role of high-throughput Strategies for increasing Crop productions

Now, the DNA sequencing has not only accelerated the biological research but a central cause of new discovery and development in all fields of living science. Genetic diversity in the germplasm is another key factor for useful breeding (Ahmad et al., 2010; Renganayaki et al., 2001) and for the complex quantitative traits association mapping is so applicable in plants (Al-Maskri et al., 2012). Genetic engineering is also widely exploited to transfer a single or multiple genes of interest within or across species. As a result, plants which are tolerant to a multitude of environmental stresses or those with improved nutritional qualities are obtained (Bhatnagar-Mathur et al., 2008). Here we have try to brief the certain High-throughput techniques that can play a central role in the development of agriculture sector in Pakistan.

1-Genome Wide Association (GWA) Studies, a green signal for agriculture sector in Pakistan

Genome-wide association study is performed by scanning, an entire genome for SNPs associated with a particular trait of interest (Gibson and Muse, 2009). Today a hundreds of GWA studies have been developed not only for the welfare of human and animals but for plants. We think that these latest techniques can also play role in the development of agriculture.

A- GWAS based on linkage disequilibrium (LD)

This technique is a best source for fine mapping of quantitative trait loci (QTL) that are responsible for basic agronomic traits (Pasam et al; 2011). Through this, Pakistani agricultural researchers can explore the accurate genetic basis of disparity for the traits like, grain weight, grain yield, plant height, starch content and crude protein content in a diverse collection that are enough for crop improvement. Currently, gene mapping efforts are shifting from conventional FBL based mapping to LD based association mapping (Goldstein and Weale, 2001). Linkage mapping (analysis) is the most common approach in plants to detect quantitative trait loci (QTL) corresponding to complex traits. While, in linkage mapping, linkage disequilibrium (LD) is generated by establishing a population from a cross between two parental lines. If we compare the linkage analysis that can only sample a small fraction of all possible alleles in a population from which the parents originated. An alternative approach, association mapping (AM) known as LD mapping relies on existing natural populations or designed populations of plants to overcome the constraints inherent to linkage mapping (Pasam et al., 2011)

Overall, We will recommend this strategy to Pakistani researchers of agriculture and Doctorate students because; it has been successfully measured in various crop species extending up to 10-50cM in soybean (Zhu et al., 2003; Jun et al., 2007), 50cM in sorghum (Hamblin et al., 2004), 10cM in sugarcane (Flint-Garcia et al., 2003), 3cM in sugerbeat (Kraft et al., 2000), 200bp in silage maize (Guillet-Claude et al., 2004), 0.5kb - 2 kb in rye grass (Skøt et al., 2005; Ponting et al., 2007; Xing et al., 2007), 5-10cM in grape (Barnaud et al., 2006) and 16-34kb in poplar (Yin et al., 2004). While in cotton (Abdurakhomonov et al., 2008) studied the LD extent who surveyed 200 SSRs in germplasm of 335 genotypes and reported up to 25 cM with $r^2 > 0.1$.

B- Hap Map an Extent of Linkage Disequilibrium (LD)

Haplotype Map is a step forward from the oligonucleotide array technology for accurate imputation in organisms commonly used for GWAS (Clark et al., 2007; Weigel and Mott, 2009). In species or population where LD decays quickly but a higher read coverage will be necessary; an obvious biological consideration that will affect genotype imputation is the extent of LD (Huang et al., 2010; Clark et al., 2010). The main objective of this technique is to determine the patterns of LD in the populations as in case of International Hap Map Project that has pre-calculated the patterns of LD among the genotype SNPs. The knowledge of the extent of LD in the region is so important for a researcher who wants to conduct a study for the detection of association between a common allelic variation of a gene and a disease of interest. Here, we will also suggest the awareness of the extent of LD for Pakistani agricultural researchers because it is an emerging approach for identifying genes underlying complex diseases at an unprecedented rate (Huang *et al.*, 2010) and also so essential for reducing the number of SNPs that need to be genotyped across the region.

2- RNA-Sequencing

Another stimulating application of HTS technologies is RNA sequencing. RNA-Seq can incorporate multiple tasks in a single channel by saving money and time. This methodology is widely accepted for the identification of alternative splice variants and for the discovery of novel transcripts (Wang et al., 2009; Howard and Heber, 2010). The technique is also useful for transcript profiling and/or SNP finding and annotation, in a number of plant species including *Arabidopsis* (Weber et al., 2007;

Wall et al., 2009), soybean (Libault, 2010; Severin, 2010), rice (Lu et al., 2010), maize (Barbazuk et al., 2007) and *Medicago truncatula* (Cheung et al., 2006). It is anticipated that on the behalf of base-pair resolution, higher sensitivity and a large range of expression values, RNA-Seq methodologies will replace the microarrays for transcript profiling (Marguerat and Bahler, 2010; Marioni et al., 2008). The significant genes or the entire metabolism network could be established through the data mining of the high throughput RNA sequencing. The genes responsible for high yield, stress tolerance or disease resistance can be obtained for functional investigation. The transgenic research using those critical genes will be the promising way in improving the production and quality of major crops.

In Pakistan, if we see the agricultural output, it is decreasing gradually as compared to many other developing countries of similar resources. The reality is that agriculture sector is not benefited completely from such kind of techniques except tissue culture technology at certain level. The level of such types of research efforts is very small, both at private and public level as compared to China. Same while, the size of these efforts is also so small that is unable to commercialize in Pakistan. The basic reason of high agricultural yield in all developed and top most developing countries is that they focused on traditional biotechnology along with modern Biotechnology like genetic engineering and plant genomics as a basic tool for applied breeding. Agricultural biotechnology research and development is suggested to focus areas that has a potential to diagnose diseases, pests control, to avoid contaminants, vaccine development, to tolerance of environmental stresses and to improved nutritional contents in crops of Pakistan.

Table 1: Yielding gap (Major crops) between China and Pakistan Source

Country	Wheat (000 Tons)	Difference from the best* (%)	Sugarcane (000 Tons)	Difference from the best* (%)	Rice (Paddy)	Difference from the best* (%)	Cotton Seed (000 bales)	Difference from the best* (%)
World	3086	65	71510	59	4309	44	2099	54
China	4762	100	73114	60	6556	67	3906	100
Pakistan	2451	52	51494	43	3520	36	2046	52

*Best = 100

Source: Ministry of Food, Agriculture and Livestock, Govt. of Pakistan

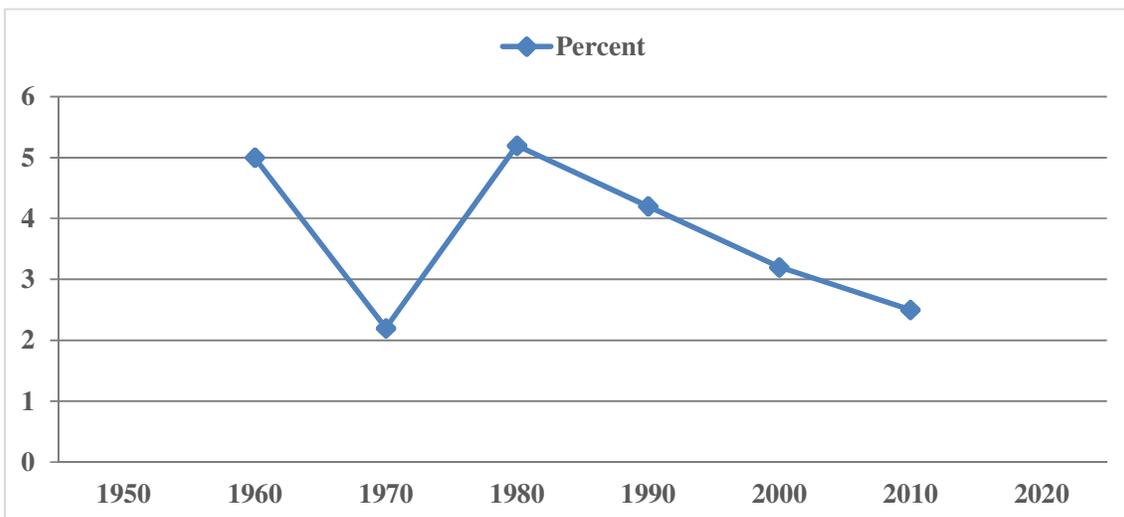


Fig 1: 65-years historical growth performance of agriculture in Pakistan (*Federal Bureau of Statistics, Pakistan*)

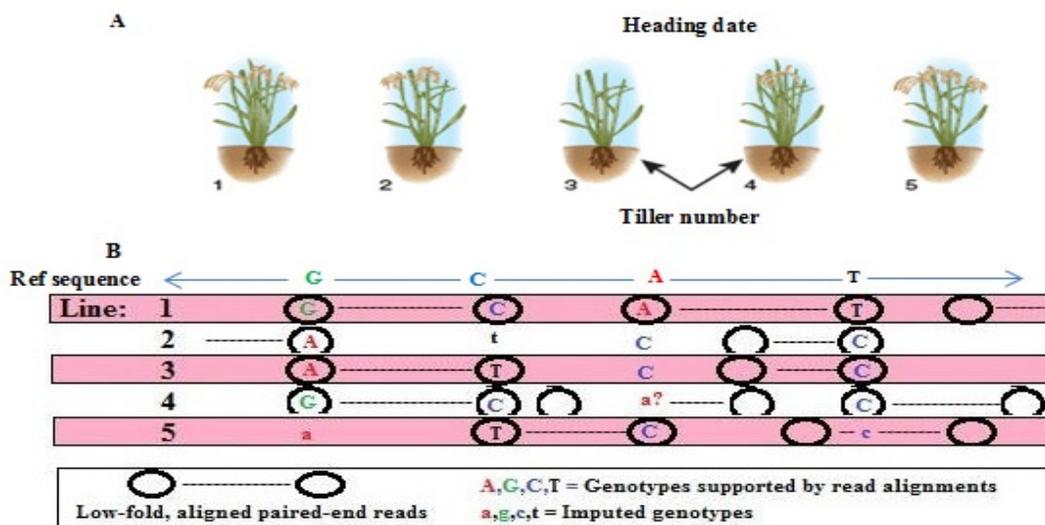


Fig 2: A sample of rice HapMap for GWAS with low-fold sequencing. (A) Lines of rice vary for agronomically important traits, such as heading date (flowering time) and tiller (branch) number. (B) Low-fold sequencing and read alignment to the reference (Ref) rice genome sequence is both efficient and sufficient for genome-wide genotyping. Aligned reads (gray boxes) enable SNP discovery in different lines (bases, upper case; consistent patterns of discrepancies in alignments distinguish genetic differences from sequencing errors). Further, imputation ‘fills in’ missing genotypes in areas of no coverage (bases, lower case), even though factors such as (historical) recombination will inherently lead to uncertainties in some regions (line 4, question mark). For simplicity, only no overlapping reads are displayed for each line. Paired-end reads 5 (gray boxes with dashed lines connecting the read pairs) were used by Huang *et al.*, 3 and facilitate correct read alignment and discovery of structural variants (but are not formally required).

CONCLUSION

Conclusively, we can say that, the advantage of modern techniques (next generation high-throughput sequencing techniques) for the analysis of genomes and transcriptomes cannot be ignore in any state (Mortazavi et al., 2008; Wang et al., 2009). It is true that the prospective for allocating more land and water resources to agricultural production and/or scope of further increase in cropping intensity are restricted in Pakistan. Moreover, the national health and environmental concerns inputs like pesticides and fertilizers also cannot be increased. Now, a possible way forward is to increase efficiency and sustainability of current crop production practices along with incorporating modern agricultural biotechnology (McMichael, 2001). There is highly need of proper management in order to preserve the natural resources in the form of soil and water for the prosperity of agriculture at national level (Khan and Hanjra, 2009). If we see at world level there are two basic things for agricultural growth: (1) the technological change that relies on research and development (Evenson, 2002); (2) the technical efficiency with which new technology is adopted and used for desired rapid growth. In our eye, agriculture mostly depends on better infrastructure, flow of information, quality inputs, availability of funds and farmers managerial capabilities. Today, the China-Pakistan friendship and strategic partnership of cooperation is in the front of whole world. All-round cooperation and harmonious coexistence between two countries is very popular so, Pakistan can take benefit from Chinese experience and expertise in agricultural technologies to ensure its food security as China is feeding around 21 per cent of the world population by just bringing four per cent of the total global area under crops. Finally, despite all these challenges and weakness, agriculture is until a major source of income in Pakistan. Agriculture is not only contributing a big share in the GDP but also a major source of export in the country. Therefore, most of the future increase in crop production must come from greater yield but not from expanding area. All in all actual research and well organized strategies can drive increased agricultural production in Pakistan.

ACKNOWLEDGEMENTS

We thank Dr. Zulfiqar Ali (University of Agriculture, Faisalabad, Pakistan) and Dr. Azeem Iqbal Khan (CABB, University of Agriculture, Faisalabad, Pakistan) for their useful suggestions and discussion.

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