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Fertiliser Best Management Practices for Maize Systems

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Maize is an important crop for food and nutritional security in India. Strong market demand and resilience of maize to abiotic and biotic stresses have increased the area and production of maize in the country over the past decade. Productivity of maize, however, has not increased proportionately and significant yield gaps are evident across maize growing areas in the country. Maize is an exhaustive crop and removes large amounts of plant nutrients from the soil to support high biomass production. The 4R Principles of applying right source of nutrients, at the right rate, at the right time and at the right place is expected to increase nutrient use efficiency, productivity and farm profit from maize production and provides opportunity for better environmental stewardship of nutrients. Adaptation of 4R Principle-based site-specific nutrient management decision support tools provides the opportunity for large-scale adoption of improved nutrient management across maize ecologies.

Maize, a crop of worldwide economic importance, provides approximately 30% of the food calories to more than 4.5 billion people in 94 developing countries. The demand for maize is expected to double worldwide by 2050. Maize is considered as the third most important food crop among the cereals in India and contributes to nearly 9% of the national food basket (7). Grown in an area of 8.55 mha with an average productivity of 2.5 t ha^{-1}, maize contributes to more than half of the coarse cereal production of the country. The annual maize production in India is about 21.7 mt with an annual growth rate of 3 to 4 % (1). Maize yields in India need to be increased significantly to sustain this growth rate to meet India's growing food, feed and industrial needs.

Area, production and productivity of maize grew impressively during XIth plan at a growth rate of 2.6, 8.2 and 4.9%, as a result of commendable response both from the producers and industries (12). Historically, the area, production and productivity growth of maize during pre-green revolution era (upto 1970) was in increasing order but it remained slow and static during for almost 2 decades of post-green revolution era. However, during past one decade (2003-2011), there has been quantum increase in area, production and productivity of maize in India (**Figures 1, 2 and 3**). Introduction of single cross hybrids in Indian maize programme since 2006 resulted in productivity enhancement of 134 kg ha^{-1} annum^{-1} in the last five years although the extent of coverage was less than 25%. Growing market demand by the feed and starch industry and increase in minimum support price from Rs. 540 q^{-1} in 2006-07 to Rs. 1175 q^{-1} in 2012-13 led to make maize as a more competitive crop and

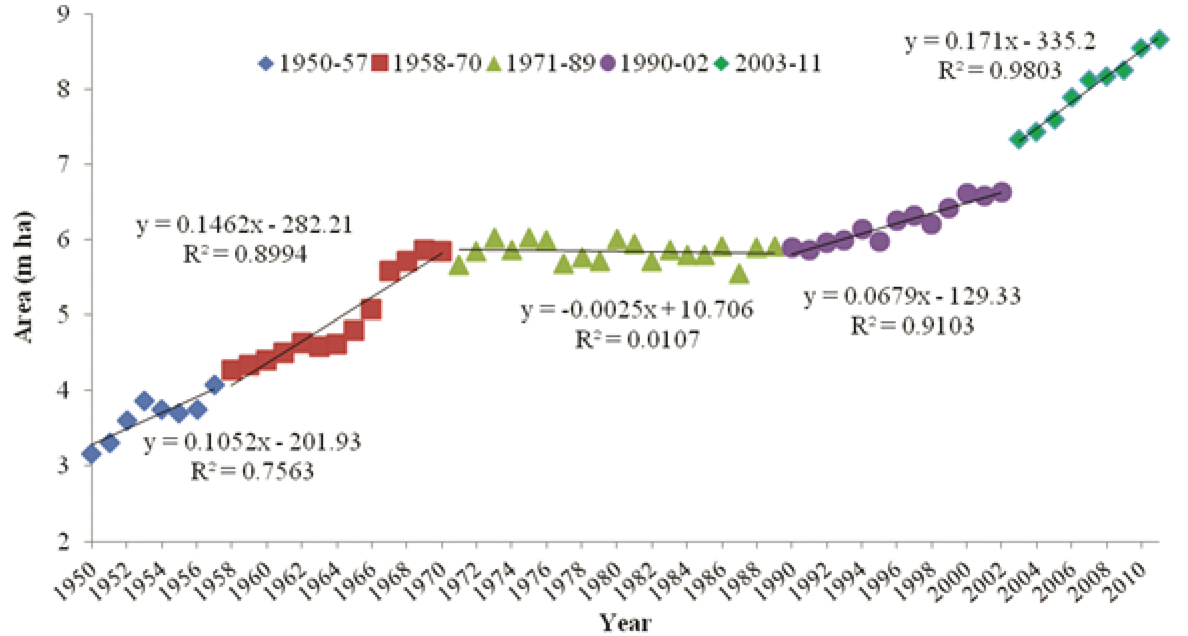

Figure 1 – Decadal trends in maize area in India during 1950-2011

encouraged farmers to grow maize to a large extent. Also, the export competitiveness of Indian maize especially for East Asian economies due to low freight charges made this crop more versatile with no carryover stocks and India exported a record 4.6 mt of maize during 2012-13 (12) and further opens the avenues for more demand of maize in India.

Consequently, maize is rapidly emerging as a favourable component crop in the major cereal based cropping systems of India. Development of high yielding maize hybrids with lesser water requirement, resilience to biotic and abiotic stresses, high resource use efficiency under various agro-climatic conditions, have led to development of new maize based cropping systems adapted to various farm typologies. With the current and projected challenges for natural resources such as water scarcity, temperature stresses etc, maize has emerged as a potential alternative for

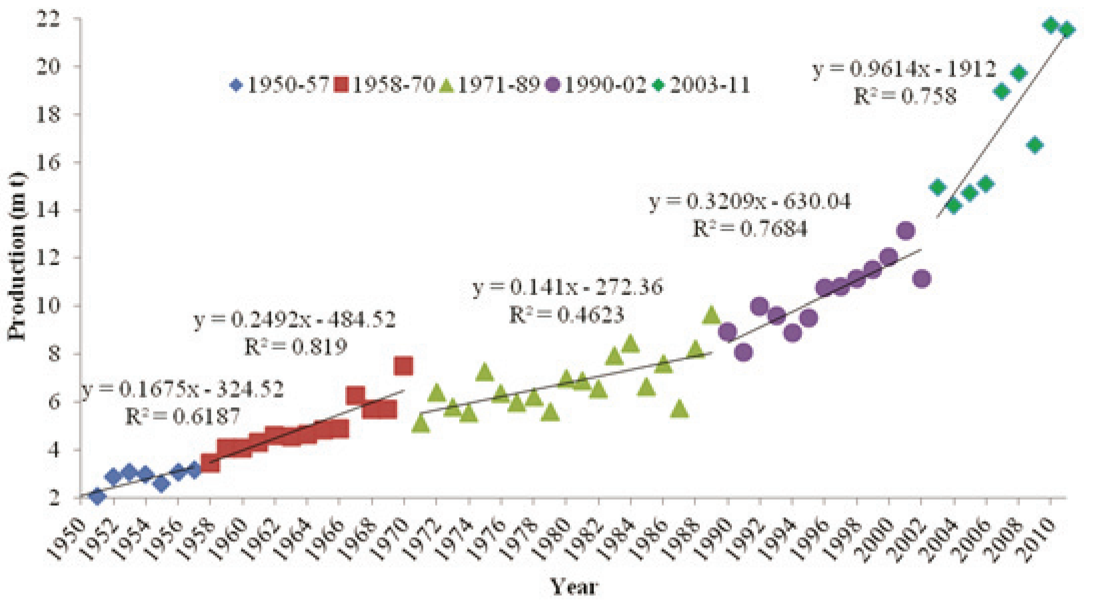

Figure 2 – Decadal trends in maize production in India during 1950-2011

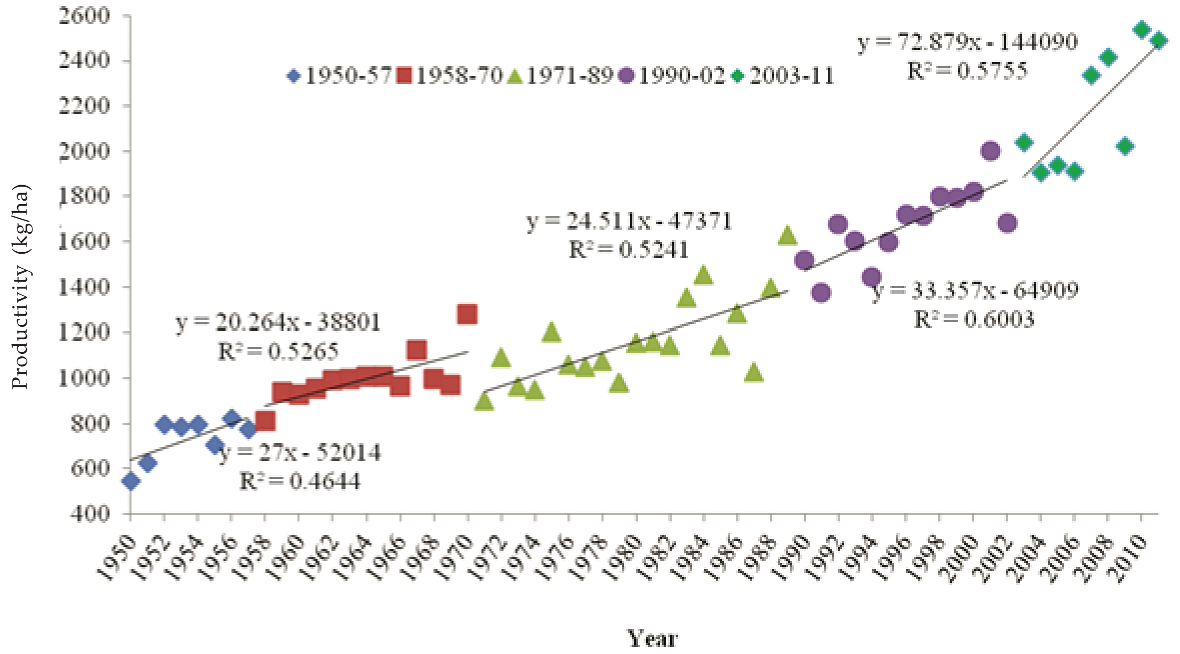

Figure 3 – Decadal trends in maize productivity in India during 1950-2011

diversification of rice-rice system with rice-maize and maize-rice systems; and rice-wheat system with rice-maize cropping systems in many ecologies of the country. Also, the market driven agriculture of specialty corn in peri-urban interface has opened another avenue for diversifying intensive cereal systems (13). High-yielding maize hybrids, with very high biomass production, extracts higher amounts of mineral nutrients from the soil than by other major cereals like rice or wheat. Biotechnology, breeding, and agronomic advancements have propelled maize yields to new highs with little guidance about fertilisation strategies for these modern maize hybrids to achieve their maximum yield potential. Being an emerging crop in many non-traditional ecologies and seasons, grown under different cropping systems and management practices, there exist large information gap on appropriate nutrient management strategies for maize in contrasting cropping systems and management practices. The fertiliser best management practices (FBMPs) for maize under such scenarios are still not well developed to help realize the sustainable benefit of these alternative maize based cropping systems. Application of existing fertilisation practices, developed decades ago, may not match uptake requirements of modern hybrids that are now grown at population densities higher than ever before. Nutrient requirement of maize varies from field to field due to high variability in soil fertility across farmer fields, and single homogenous nutrient recommendations may not be very useful in improving maize yields. Increasing fertiliser prices and escalating fuel prices in international market will make fertiliser input one of the costliest in agriculture. Fertiliser best management practices, with due importance to indigenous sources of nutrients such as organic manures, biofertilisers, crop residues, inclusion of legumes, use of nutrient efficient genotypes etc., will be required for sustainable management of emerging maize systems in the country. This paper provides a synthesis of current information on maize production systems, pros and cons of existing nutrient management strategies and the fertiliser best management practices for bridging yield gaps in current and emerging maize systems in the country.

Maize-based Cropping Systems in India

Maize is a versatile crop adapted to range of ecologies, seasons and regions in the country, and is grown in sequence or as companion crop with a range of crops under different production systems. However, the geographical spread of different maize-based rotations varies primarily with adaptability for a cropping window under prevailing ecology, land topography, soil type, moisture availability, and markets. Traditionally being a monsoon season crop, maize-wheat is still the predominant maize based system (1.8 mha) and is 3rd major crop-rotation in India and contributes ~3.0 % in national food

basket. The other major maize-based systems are maize-fallow, maize-mustard, maize-chickpea, maize-maize, maize-potato, etc. In recent past, the challenges of water shortages, temperature stresses in rice primarily in rice systems and to some extent in wheat systems, and opportunities of higher productivity of maize under these constrained environments as well as market opportunities for maize have led to evolution of several maize systems in non-traditional maize ecologies. For example rice-maize (~0.5 mha) has emerged as a potential maize system replacing winter rice in water scarce areas of double rice ecologies and wheat in terminal heat prone shorter wheat season ecologies. Introduction of high yielding maize hybrids for spring season and its adaptability to intensive rice systems have also led to evolution of rice-potato-maize system in larger areas of IGP. Emerging challenges of water scarcity and availability of very high yield potential hybrids for monsoon season coupled with improved agronomic management practices are leading to opportunities for re-evolution of maize-wheat-mungbean rotation in non-traditional intensive rice-wheat systems in western Indo-Gangetic Plains (IGP). Also, the emerging market opportunities for specialty corn and green cobs, high value intercropping systems involving maize are also emerging as potential maize systems in peri-urban regions of the country.

Yield Gaps in Maize

Fundamentally, yield gaps are caused by deficiencies in the biophysical crop growth environment that are not addressed by agricultural management practices. Yield potential (Yp) of any crop cultivar/hybrid for a site and for a given planting date is the yield achieved when grown in environments to which it is adapted, with nutrients and water non-limiting and pests and diseases effectively controlled (10). Timsina *et al.* (37) using hybrid maize models, estimated yield potential of four maize hybrids in India that ranged from 7.1 to 19.7 t ha^{-1} and reported that planting during August to November gave exceptionally high yields due to low temperature during grain filling, long growth duration, and large receipts of solar radiation at some of the locations.

Attainable yield (Yat), generally set at 80–90% of Yp, is average grain yield in farmers' fields with best management practices and without major limitations of water and nutrients. Attainable yield can be limited by variety, planting density, water and nutrient management, soil-related constraints (acidity, alkalinity, salinity, etc.), and climate-related constraints (flooding, drought, etc.). Actual yield (Yac) is the yield farmers receive with their average management under all possible constraints. The difference between attainable yield (Yat) and actual yield (Yac) of crop species and varieties can be quite large. Attainable yield of maize in farmers' fields, achieved under optimal conditions, can vary significantly across the agro-ecologies mainly due to genotype x environment interactions but also due to confounding influence of biotic and abiotic stresses and agronomic management. Dass *et al.* (6) reported Yat and Yac of maize from experiments conducted in 13 representative locations in various agro-environments for 9 years (1995–2003) under the All India Coordinated Research Project (AICRPM) on maize. The selected locations were first divided into two categories: locations having lower productivity than the national average (Banswara, Udaipur, Godhra, Varanasi, Kanpur and Chhindwara) and locations (Mandya, Arbhavi, Ludhiana, Dhaulakuan, Bajaura, Dholi and Hyderabad) with greater productivity as compared to national average. Data indicated that the Yac is always less than Yat under all the agro-environments due to limited availability of agronomic inputs and their scheduling. Potential for improving Yat was more at the locations of the first group as compared to the locations of the second group. Except Banswara, other locations of the first group showed the potential for achieving Yat of 4–6 t ha^{-1}, while Yac at all the locations of this group was less than half (1-2 t ha^{-1}) of the Yat. It has also been reported that present average Yac at farmers' fields is only about 50% of the Yat, which could be increased through adoption of improved technology. On the other hand, Yat for most locations was about 4.0 t ha^{-1} except for Arbhavi (5.9 t ha^{-1}) in the high productivity group, whereas, Yac at most of the locations of this group was more (1.2–3.4 t ha^{-1}) as compared to the low productivity group (6). The data reveal that Yat of maize can be quite large, and so yield gap between Yp and Yat, between Yat and Yac, and that between Yp and Yac can be minimized.

Systematic analysis of the role of general and location specific determinants of maize yields may help to narrow down the yield gap at various levels and improving actual yields. Potential, attainable and actual yields of maize were evaluated at seven representative locations in South Asia under various agro-environments to generate the productivity scenario of maize under these ecolologies. The analysis of the simulated, attainable and actual maize yields in major maize growing ecologies across South Asia (**Figure 4**) revealed wide 'management yield gaps' ranging from 36 to 77% (30). These gaps are ascribed mainly to three major factors, (i) low yielding genotypes, (ii) poor crop establishment due to random broadcasting and (iii) inadequate and inappropriate fertiliser nutrient applications as 15-45% maize acreage remains un-fertilised and the rest of the acreage has imbalanced nutrient applications (6, 15).

Nutrient Use in Maize

Nutrient removal is far excess of their replenishment under intensively cropped cereal systems in India, which has led to widespread multi-nutrient deficiencies in soils, consistently increasing response of crops to nutrient application (21). As a result of improved agronomic, breeding, and biotechnological advancements in maize systems, yields have reached far higher levels than achieved ever before. However, greater yields of maize have always been accompanied by a significant removal of macro and micronutrient from the soil. The latest summary on soil test levels in North America by IPNI reported that an increasing percentage of U.S. and Canadian soils have dropped to levels near or below critical P, K, S, and Zn thresholds during the last 5 years (11). Soils with decreasing fertility levels, coupled with higher yielding hybrids, suggest that farmers have not sufficiently matched nutrient uptake and removal with accurate maintenance fertiliser applications. Timsina and Majumdar (36) indicated that maize grain yields in Bangladesh have been decreasing where maize was grown on the same land for the last 5 to 10 years. The authors attributed the yield decline to imbalanced and inadequate nutrient application by farmers.

Maize with the yield potential of less than one t/ha removes about 90-100 kg/ha of nutrients from the soil **(Table 1)**. With the introduction of improved cultivars, the productivity has increased up to 4.0 t/ha with nutrient removal of around 220 kg/ha. Introduction of single cross hybrids, the productivity further increased to 7.0 t/ha and total nutrient removal has also increased to 420 kg/ha.

In several states of the country particularly hill ecologies (North Eastern Himalayas, Uttarakhand, Himachal Pradesh) and rainfed and tribal states (Madhya Pradesh, Chhattisgarh, Rajasthan, Orissa, and West Bengal), large area under maize production still remained untreated with fertilisers. The extent of area was up to 90 % especially in areas where farmers are not sure to harvest their crop due to abiotic stresses, particularly during monsoon season. Besides, the current nutrient use in the high input maize systems indicates imbalance plant nutrition with very high use of N and less use of P and negligible use of K fertilisers and micro nutrients. This has led to nutrient imbalances in soils and lower nutrient use efficiency and economic profitability. This warrants adequate and balanced use of plant nutrients not only for specific farm and ecology but also in production systems using fertiliser best management practices adapted to local situations and farm typologies to achieve better efficiency and nutrient stewardship.

Nutrient Response of Maize

While managing plant nutrients in maize systems, nitrogen (N), phosphorus (P), and potassium (K) remain the major ones for increased and sustained productivity. However, cultivation

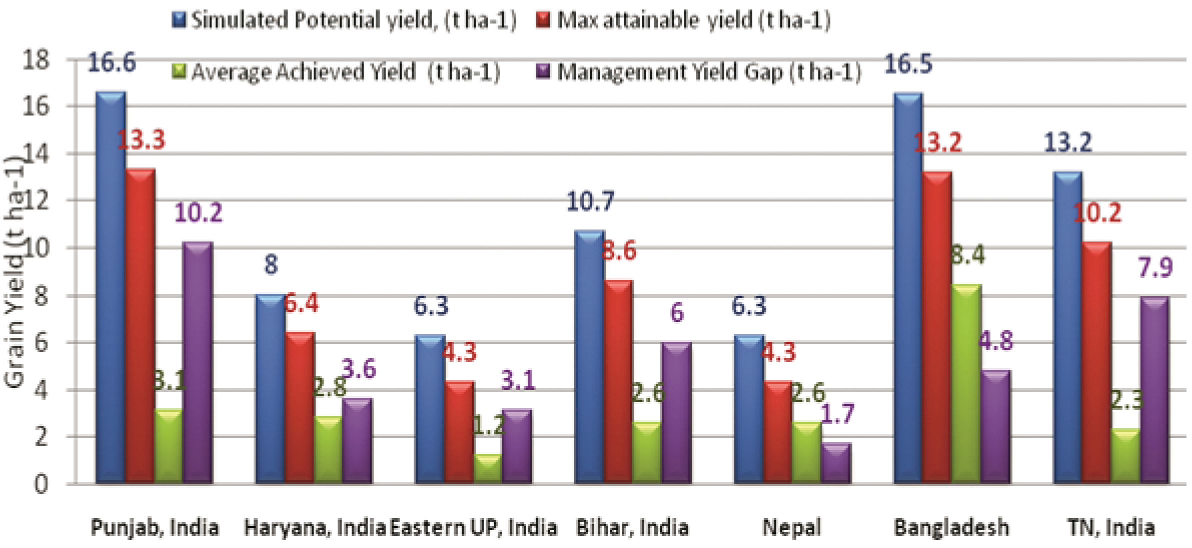

Figure 4 – Potential, attainable and actual yields and management yield gaps under different ecologies across South Asia

Table 1 – Major nutrient removal from the soil by the maize plant

Plant part	Yield (t/ha)	Nutrient extraction (kg/ha)						Total
		N	P	K	Ca	Mg	Zn	
Traditional cultivars								
Grain yield	1.0	25	6	15	3.0	2.0	0.023	51.0
Stover	1.5	15	3	18	4.5	3.0	0.040	43.5
Total	2.5	40	9	33	7.5	5.0	0.063	**94.5**
Improved cultivars								
Grain yield	4.0	63	12	30	8.0	6.0	0.093	119.1
Stover	4.0	37	6	38	10.0	8.0	0.108	99.1
Total	8.0	100	18	68	18.0	14.0	0.201	**218.2**
Hybrid cultivars								
Grain yield	7.0	128	20	37	14.0	11.0	0.163	210.2
Stover	7.0	72	14	93	17.0	13.0	0.189	209.2
Total	14.0	200	34	130	31.0	24.0	0.352	**419.4**

Source: (41).

of high yielding maize systems will likely exacerbate the problem of secondary and micronutrient deficiencies, not only because larger amounts are removed, but also because the application of large amounts of N, P, and K to achieve higher yield targets often stimulates the deficiency of secondary and micronutrients (17). However, for determining right rates of nutrients, information on crop yield response to fertiliser application, agronomic efficiency and return on investment (ROI) to fertiliser application is also essential. Soils of the major maize growing areas in India are inherently low in soil organic matter and nitrogen is the major limiting plant nutrient, with N availability being routinely supplemented through application of fertilisers. Though the yield increase in maize due to N fertilisation was substantial (92%), the average agronomic efficiency of N (kg grain kg^{-1} N) in maize was only 12.5 (28), indicating low N use efficiency. Satyanarayana *et al.* (32) reported variable maize yield response to N fertiliser application, ranging from 400-5160 kg ha^{-1} with an average response of 2154 kg ha^{-1}. Though N plays an important role in governing the yield of crops, lack of awareness on improved strategies of N management, coupled with relatively lower prices of N fertilisers (especially urea), encourages imbalanced use by farmers. Therefore, N management strategies that consider the yield response, agronomic efficiency of N (AEN), coupled with appropriate timing and splitting, may be used not only for minimizing the losses of N from agricultural fields but also for increasing the yield and profitability from N use. A recent study (3) reported that increasing N levels from 130 to 390 kg N ha^{-1} resulted in increasing maize yield from 4.3 to 9.02 t ha^{-1} in the maize-wheat cropping system (MWCS) of northern Karnataka. However, they also reported that in addition to crop response, AEN and ROI also need to be considered while deciding N application rate in the MWCS.

P response is highly variable and is influenced by soil characteristics and growing environment of the crop. P application rate, therefore, must be based on expected response of a particular location. An average maize P response of 853 kg ha^{-1} across 36 locations in Bihar and West Bengal was reported (14), which also indicated that the average P responses were higher in winter maize (1070 kg/ha) than in spring maize (513 kg/ha). However, P application based on yield response alone does not take into account the nutrient removal by crops where response is low or negligible. In such scenarios, nutrient removal by the crop would not be replenished adequately by external application, which may lead to nutrient mining and decline in soil fertility. One way to counter that would be to apply a maintenance dose that replenishes part of the nutrient exported out of the field with harvested crop part (grain and straw). This will ensure that soil fertility levels that can support intensive production systems are maintained. Finally, management of P fertiliser for maize systems must take account of residue and organic amendments applied to the soil.

Indian soils, despite often having relatively large total K content, resulted in variable yield and economic loss to the farmers with skipping application of K. Long-term use of N and P in the absence of K illustrates the seriousness of nutrient imbalance of a region. Li

et al. (19) demonstrated the effect of K fertilisation on maize production within a region that has typically relied on N and P alone. They reported that balanced use of N, P, and K fertiliser generated an average yield increase of 1.2 t/ha and improved farm income by USD 300/ha when compared to common farmer practice. Grain yield response to fertiliser K is highly variable and is influenced by soil, crop and management factors. Majumdar *et al.* (21) reported that the average yield loss of maize in Indo-Gangetic Plains due to omission of K application was 700 kg ha^{-1}. These observations were in contrary to the general perception that omitting potash for a season will not adversely affect maize production in the country. The results also demonstrate clearly the low K supply levels of most maize growing soils in India. Therefore, improved K management will have great potential for improving the overall productivity of maize systems in India.

A systematic approach of nutrient management (22) indicated that N, P, K, and Zn were the most limiting nutrients for maize growth in Tamil Nadu and relative yields were 57, 63, 71, and 75% of the optimum when N, P, K, and Zn were omitted. Also, maize yields and responses to applied nutrients varies considerably across farmer fields, mainly because of small and marginal landholdings that result in high variability in soil nutrient availability over small distances (33). The generally high variability in maize nutrient responses across fields and establishment practices suggests that spatial and temporal differences of nutrient availability needs to be accounted for while formulating nutrient management strategies in maize. Besides, large variability in crop response to all nutrients indicates the need to develop fertiliser recommendation tools that consider more than just a soil test (19). In other words, best-bet approaches for nutrient management like site-specific nutrient management and decision support tools like Nutrient Expert for Hybrid Maize, based on realistic estimates of indigenous nutrient supply and nutrient requirements for a targeted crop yield for individual farmers' fields, will be required to improve yield and nutrient use efficiencies in maize production systems.

Fertiliser Best Management Practices for Maize

Nutrient management in multiple cropping systems is a complex process. Maize and maize-based systems involving cereals, extract large amounts of mineral nutrients from the soil due to large grain and stover yields. Proper nutrient management of exhaustive maize-based systems should aim to supply fertilisers adequate for the demand of the component crops and apply in ways that minimize loss and maximize the efficiency of use. The amount of fertiliser required depends on many factors including the indigenous supply of each nutrient which can be of appreciable quantities (5). Phosphorus inputs from irrigation and rain waters are negligible but 1,000 mm irrigation through surface water may provide up to 30 kg K ha^{-1} yr^{-1} (8, 9) and up to 1,100 kg S ha^{-1} yr^{-1} (27). In Rice-Maize (RM) systems, K inputs may be much larger than 30 kg ha^{-1} where groundwater is used. Thus, emphasis must be upon the nutrient requirements for target yields and nutrient supply by integrated use of indigenous sources, soil organic matter (SOM), farm yard manure (FYM), composts, crop residues, and increasingly, fertilisers to achieve and sustain high yields and nutrient use efficiencies of intensive maize-based systems. Fertiliser is the dominant source of nutrients and is required to increase yield of crops but should be applied in such a quantity that it becomes profitable and will have least adverse effect on environment.

Improving our understanding of uptake timing and rates, partitioning, and remobilization of nutrients by maize plants provides opportunities to optimize fertiliser rates, sources, and application timings. Optimizing nutrient management in maize systems includes using the right source at the right rate, at the right time, and at the right place - the 4R approach (4). While developing fertiliser recommendations for maize, two major aspects of plant nutrition are important to understand for managing high yielding maize production systems. This includes: 1) the amount of a given mineral nutrient that needs to be acquired by the plant during the growing season, referred to as "total nutrient uptake," or nutrients required for production, and 2) the amount of the nutrient transported out of the field with grain and straw/stover, referred to as "removed with harvested product". Providing the nutrients as and when required by the crop and replenishing the exported nutrient out the field with harvested products ensures sustainability of production systems. Further improvement of fertility practices require matching in-season nutrient uptake with availability, a component of the right source, which is interconnected with the other components of 4R Principle. The maximum rate of nutrient uptake coincides with the greatest period of dry matter accumulation during vegetative growth for most nutrients. Unlike the other nutrients, P, S, and Zn accumulation are greater during grain-filling than vegetative growth; therefore, season-long supply is critical for balanced crop nutrition. Similarly, micronutrients demonstrate more narrow periods of nutrient uptake than macronutrients, especially Zn and B. Therefore, fertiliser sources that supply nutrients at the rate and time that match maize nutritional needs are critical for optimizing nutrient use

efficiencies.

Effectively minimizing nutrient stress requires matching nutrient supply with plant needs, especially in high-yielding conditions. Sulphur and N, for example, are susceptible to similar environmental challenges in the overall goal of improving nutrient availability and uptake. However, the timing of N uptake in comparison to S is surprisingly different (2), suggesting practices that are effective for one but may not improve uptake of the other. In case of Nitrogen, two-thirds of the total plant uptake is acquired by VT/R1 crop physiological stage of maize, whereas S accumulation is greater during grain-filling stages with more than one-half of S uptake occurring after VT/R1. Similarly, potassium, like N, accumulates two-thirds of total uptake by VT/R1 and greater than one-half of total P uptake occurs after VT/R1 (2), suggesting that season-long supply of P and S is critical for maize nutrition while the majority of K and N uptake occurs during vegetative growth. Unlike N, P, K, and S, which have a relatively constant rate of uptake, micronutrients exhibit more intricate uptake patterns. Uptake of Zn and B, for example, begins in the early vegetative stages and reaches a plateau at VT/R1 stage of the crop. Thereafter, Zn exhibits a constant uptake rate similar to that of P and S, while B uptake follows a major sigmoidal uptake phase concluding around R5 stage of maize. Zinc and B follows shorter periods of more intense uptake in comparison to macronutrients. Late vegetative and reproductive growth, constituting only one-third of the growing season, accounts for as much as 71% of Zn uptake by maize. A similar trend is also noticed for B where, as much as 65% of B uptake occurred over only one-fifth of the growing season (2). This also indicates that matching micronutrient needs of maize in high- yielding conditions clearly requires supplying nutrient sources and rates that can meet crop needs during key growth stages. Therefore, the 4R approach (right source, right time, right amount and right place) holds merit not only attaining higher yields but efficiency, profitability and environmental stewardship.

Integrated Nutrient Management Including Crop Residues

Intensified and multiple cropping systems require judicious application of fertiliser, organic and bio-fertilisers for yield sustainability and improved soil health. Integrated plant nutrient supply (IPNS) system encompasses a combined use of different sources of plant nutrients for maintaining and improving the soil fertility for sustainable crop production without degrading the soil resource on long-term basis. It relies on a combined use of organic manures including green manures, recycling of crop residues, bio-fertilisers, vermicompost and a judicious and need based use of fertilisers. A summary of multi-location trials on integrated nutrient management in maize (16) under partially irrigated conditions (**Table 2**), comprising of different combinations and levels of organic and fertiliser sources of nutrients {without organic manure (O_0) and application of FYM @ 6 t/ha (O_1) with four levels of fertiliser nutrients *i.e.,* 100:40:30 (N_1), 150:60:40 (N_2), 187:75:50 (N_3) and 225:90:60 N: P_2O_5: K_2O kg/ha (N_4)}, showed that application of FYM @ 6 t/ha at N_4 level resulted in highest grain yield during both the years which was at par with sole fertiliser application at N_3 level in the second year. The application of O_1N_4 resulted in 21.5 & 25.2; 14.4 &13.6; 9.2 & 11.6; 20.0 & 16.8 and 11.1 & 9.0 per cent increase over O_0N_1 , O_0N_2, O_0N_3, O_1N_1, and O_1N_2 in the pooled grain yield of all the locations during 2007 and 2008, respectively. Pooled analysis of nutrient productivity across locations during both the years showed that it was highest with the application of O_0N_1 treatment as the application level produced maximum yield response often observed at the lower part of yield response curves.

While managing maize residues in IPNS, it is important to understand nutrient distribution within the maize crop. Of the total nutrient

Table 2 – Effect of integrated nutrient management on yield and nutrient productivity (kg/ha) of quality protein maize in different agro-ecologies (pooled data of 7 locations across different ecologies in India)

Treatment	Yield (kg/ha)		Nutrient productivity (kg grain/kg nutrient applied)	
	2007	2008	2007	2008
O_0N_1	4226^{f}	4395^{f}	28.1^{a}	26.1^{a}
O_0N_2	4735^{e}	4930^{de}	20.8^{b}	20.5^{b}
O_0N_3	5136^{cd}	5173^{cd}	17.7^{bcd}	16.8^{c}
O_0N_4	5482^{b}	5512^{bc}	15.4^{cd}	14.7^{cd}
O_1N_1	4482^{ef}	4766^{ef}	19.9^{bc}	20.1^{b}
O_1N_2	5069^{d}	5333^{c}	16.6^{bcd}	16.6^{c}
O_1N_3	5433^{bc}	5745^{ab}	14.9^{d}	14.6^{cd}
O_1N_4	5839^{a}	6099^{a}	13.5^{d}	13.2^{d}
p value	<.0001	<.0001	<.0001	<.0001

Means with at least one letter common are not statistically significant using Fisher's Least Significant Difference

uptake in maize, nearly 80% of P is removed in maize grain compared to K and B, which are retained to a greater percentage in stover. For each nutrient, the fraction that is not removed with the grain remains in leaf, stalk, and reproductive tissues and constitutes the stover contribution that is returned to the field if the residues are returned back into the field. Returning the stover recycles back about 25% of N and P, 50 % of S and 75 % K uptake by cereal crops and replenishes large part of nutrient off-take from the field. Effective stover management through conservation agriculture based management practices can ensure almost 50% nutrient and most of the potassium and micronutrients back to the soil which will ensure sustainability of maize production in future. However, the availability of such nutrients, immediately after recycling of the straw, is influenced by microbial immobilization and mineralization processes and may not meet the nutrient demands of high yielding maize at the time of rapid growth stages.

Site-specific Nutrient Management (SSNM)

Precision Agriculture is an emerging concept wherein the input variables such as fertilisers are applied in right amount, at the right place and at the right time (variable rate application) as per demand of the crop-plants, rather than prophylactic application. It helps to improve input use efficiency, economy, and ensures sustainable use of natural resources, as it minimizes wastage. Site-specific nutrient management (SSNM) is one such approach that utilizes FBMPs for optimizing nutrient management in crops, including maize.

Site-specific nutrient management is a widely used term in all parts of the world, generally with reference to addressing nutrient differences, which exist within and between fields, and making adjustments in nutrient application to match these location or soil differences (17). In brief, the use of any diagnostic tool to evaluate soil fertility status, and subsequent prediction of external nutrient supply based on a specific crop yield goal, became the practice associated with the use of the term SSNM. It describes nutrient management recommendations that take into account the soil, crop to be grown and growing conditions of a specific location. SSNM recommendation may varies significantly, from a field specific soil test to output of decision support models based on predictive equations supported by SSNM principles. Ultimately, the success of a SSNM recommendation can be judged based on its performance relative to either a state recommendation, or the existing farmers' practice. Whether we have increased productivity and profitability to the farmer as compared to existing practices, and addressed the efficiencies necessary to support the sustainable use of fertiliser nutrients, defines the success of an SSNM approach. The SSNM approach was successfully implemented by the International Plant Nutrition Institute (IPNI) that improved field specific recommendation to a farmer, in a cost effective and timely fashion (18).

An experiment was conducted in rice-maize system at AICRP on maize centre Hyderabad, comparing SSNM with that of state recommendation and recommendation based on AICRP results, showed that highest yield of both rice and maize and also the highest system productivity were obtained with SSNM (35). This study further indicated that application of SSNM principles, aided by nutrient balance studies, can help improve nutrient management in rice-maize systems towards improving yield and profitability (**Figure 5**). Another field experiment on SSNM, conducted under AICRP on Maize in two major maize-based cropping systems, *i.e.* maize-wheat at 8 locations (Delhi, Bajaura, Udhampur, Dholi, Ludhiana, Pantnagar, Banswara and Ranchi) and rice-maize at 3 locations (Jorhat, Banswara, Hyderabad) during *Kharif* 2008 (**Figure 6**), indicated a significantly higher yield of maize under SSNM compared to state recommendations at most of the locations.

Nutrient Expert Decision Support System for SSNM in Maize Systems

SSNM is, however, a knowledge-intensive technology in which optimum fertiliser management for a crop field is tailored to specific local condition, growth duration of the variety, crop residue management, past fertiliser use, and input of nutrients from external sources. Such knowledge requirements have slowed the wide-scale promotion and adoption of SSNM by the farmers. Development of tools that consolidate the complex and knowledge-intensive SSNM information into simple delivery systems is the key for enabling farmers and their advisors to rapidly implement this technology on a large scale. IPNI in collaboration with CIMMYT has recently developed Nutrient Expert (NE), a new nutrient decision support system (DSS) for maize, based on SSNM principles. Nutrient Expert, while providing fertiliser recommendations, considers yield response and targeted agronomic efficiency in addition to the contribution of nutrients from indigenous sources. It also considers other important parameters of the growing environment affecting nutrient management recommendations in a particular location and enables crop advisors to provide farmers with fertiliser guidelines that are suited to individual farming

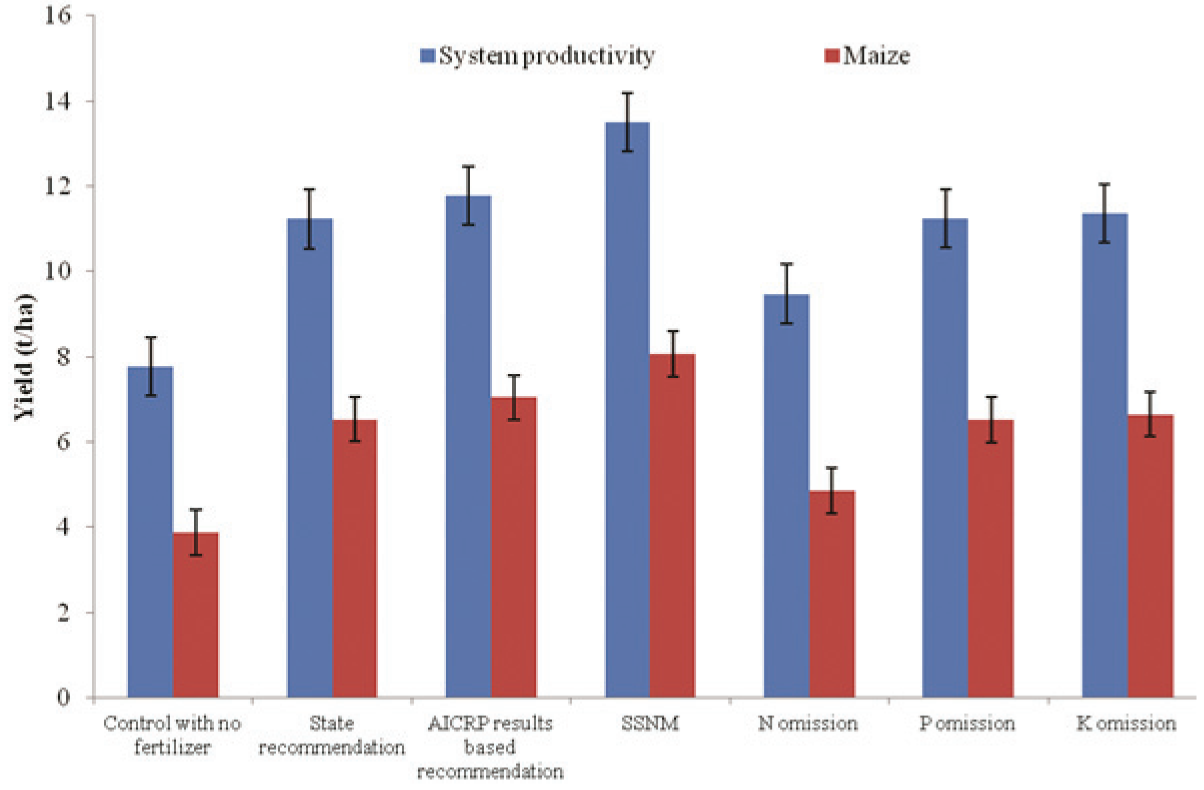

Figure 5 – Grain yield of maize and system productivity of an SSNM experiment in rice-maize system

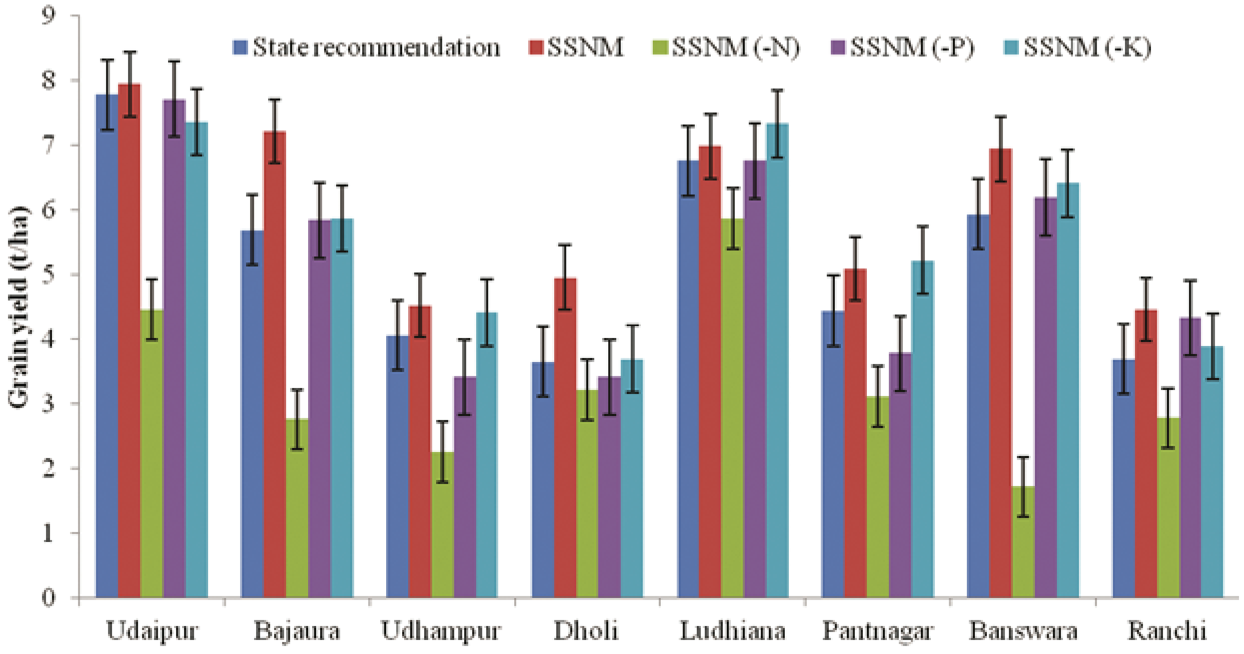

Figure 6 – Effect of nutrient management practices on grain yield of maize at different locations in India

Table 3 – Comparison of nutrient use in maize between NE and FP in southern India during Kharif 2011

Parameter	Unit	FP	NE	NE-FP	
		Southern India (n = 32)			
Fertiliser N	kg/ha	80-550 (193)	110-230 (161)	-32	Ns
Fertiliser P_2O_5	kg/ha	38-230 (89)	17-81 (39)	-50	***
Fertiliser K_2O	kg/ha	23-352 (114)	18-104 (48)	-66	***

***Significant at $p < 0.001$; Ns = non-significant.FP, and NE = Farmer Practice, and Nutrient Expert. Values in parenthesis represent mean values

conditions. The tool uses a systematic approach of capturing site information that is important for developing a location-specific recommendation (24). The tool has been successfully used to provide farmer specific fertiliser recommendations in the major maize growing ecologies across the country and improved yield and farmer profit as compared to existing fertiliser management practices. A recent study using the NE tool for maize in South India (31) revealed that the N, P_2O_5, and K_2O use by farmers varied from 80 to 550, 38 to 230, and 23 to 352 kg/ha, with an average of 193, 89, and 114 kg/ha, respectively. The corresponding NPK use based on NE recommendations varied from 110 to 230, 17 to 81, and 18 to 104 kg/ha, with an average of 161, 39, and 48 kg/ha, respectively. The NE-based fertiliser recommendations reduced N, P_2O_5, and K_2O use by 32, 50, 66 kg/ha indicating 17, 56, and 58% reductions in fertiliser use over farmers' practice (FP). Data in **Table 3** for nutrient use in Kharif maize further revealed that the lowest N use in FP has increased from 80 to 110 kg/ha in NE, whereas, the maximum N use in FP has decreased from 550 to 230 kg/ha in the NE based recommendations. Data pertaining to relative performance of NE over sate recommended fertiliser dose (SR) and FP for grain yield of maize, fertiliser cost, and GRF in the same study are given in **Table 4**. Across all sites (n=32) during the Kharif season, NE-Maize increased yield and economic benefit (i.e. gross return above fertiliser costs or GRF) over FP and SR (**Table 4**). Compared to FP, it increased yield by 1.06 t/ha and GRF by 12,902 INR/ha with a significant reduction in fertiliser cost of 3,239 INR/ha. Recommendations from NE-Maize also increased yield (by 0.9 t/ha) and GRF (by 8,033 INR/ha) over SR with a moderate reduction in fertiliser cost (-1,041 INR/ha). This indicates that NE, in addition to suggesting the right rate of nutrients sufficient to meet the attainable yield targets, also helps in optimising nutrient use through appropriate reductions in fertiliser application. In contrast to SR, which gives one recommendation per state (e.g. 150 kg N, 75 kg P_2O_5, and 75 kg K_2O per ha in Andhra Pradesh), NE recommended a range of N, P_2O_5, and K_2O application rates depending on attainable yield and expected responses to fertiliser at individual farmers' fields. Further, the estimated maize yield response by NE to application of N, P_2O_5 and K_2O fertilisers across the growing seasons varied from 2 to 8, 0 to 1.8, and 0 to 2 t/ha with a mean response of 5.02, 0.69, and 0.77 t/ha (data not shown), and captured the temporal variability of nutrient requirement between seasons along with the spatial variability between farmers' fields. The varied yield response to N, P, and K application suggests that single homogenous state recommendations may become inadequate for improving maize yields in the region. Thus, fertiliser N, P_2O_5, and K_2O requirements determined by NE, varied among fields or locations, proved to be critical in improving the yield and economics of maize farmers in the region. In effect, use of the NE actually increased yields and profit, while reducing economic risk to the farmer, simply by

Table 4 – Performance of NE based recommendations for yield and economics of maize in southern India

Parameter	Unit	Kharif 2011 (Monsoon season)				
		FP	SR	NE	NE-FP	
		Southern India (n = 32)				
Grain Yield	kg/ha	6874	7033	7936	1062	***
Fertiliser Cost	INR/ha	7214	5016	3975	-3239	***
GRF	INR/ha	61484	66353	74386	12902	***

*** Significant at $p<0.001$, GRF = gross return above fertiliser cost;
SR-State Recommendation Prices (in INR/kg): Maize = 10.00; N = 11.40; P_2O_5 = 32.2; K_2O = 18.8

providing some direction in the most appropriate fertiliser rate.

Yield improvement with NE-based fertiliser recommendation could primarily be attributed to a balanced application of nutrients based on SSNM principles. The NE program recommended application of secondary and micronutrients especially S, Zn, Mn, Fe, and B at 24 out of 32 locations in the study area (data not shown). This clearly explains how NE helped in promoting balanced use of all the essential nutrients thereby improving yields and optimising nutrient use in the maize growing areas of Southern India.

Other Precision Tools and Techniques for Real-time Nutrient Application

Blanket recommendations based on fixed-time application of fertiliser N doses at specified growth stages do not consider the dynamic soil nutrient supply and crop nutrient requirements, and lead to untimely application of fertiliser nutrients. Therefore, need based fertiliser management in maize can help to improve recovery efficiency and to reduce nutrient losses. In-season N application adjustments of maize can be accomplished using leaf colour charts (LCC), SPAD and Green-Seeker sensors. Improved N management using the LCC has consistently shown to increase yield and profit as compared to FFP (29). Applying right rate of N (240 and 150 kg/ha in maize and wheat), coupled with the right timing for N fertiliser (3-split applications) using LCC-based real time N management proved to be beneficial in increasing the yield and profitability of maize-wheat farmers of Northern Karnataka (3). Singh *et al.* (34) evaluated different need based fertiliser N management strategies in maize and confirmed the usefulness of LCC 5 as threshold during vegetative growth stages for improving fertiliser N recovery efficiency and for obtaining high yields. They also observed that there was no response to fertiliser N application at R1 stage following different LCC threshold values. The authors further recorded that using LCC 5 as threshold of N application led to equivalent grain yield achieved with fixed time application of 150 kg N ha^{-1} but with the application of only 90 kg N ha^{-1}. The recovery efficiency was increased by 19.8–22.8 along with grain yield production improvement by 7.1–8.5 kg grain per kg applied fertiliser N.

Other Issues

There are other important crop management strategies that have positive influence on FBMPs.

Cropping System Optimization Including Legumes in Maize Systems

Optimizing cropping systems is one of the best-bet management strategies not only for improving productivity but also profitability and resource use efficiency and nutrient economy. In India, several best-bet maize based leguminous systems have been identified (for example, maize- wheat-greengram, maize-maize-cowpea/greengram), which provides both the economic produce as well as stover for incorporation, as a viable means for N economy. Direct and residual effects of different legumes and sesbania green manuring on productivity, profitability, N-use efficiency and residual soil fertility in four maize based cropping systems (maize–wheat-moongbean, maize-mustard-moongbean, maize-maize-sesbania and maize-chickpea-sesbania) under conservation agriculture practices is under investigation at the Directorate of Maize Research (DMR), New Delhi, since Kharif 2008. The legumes were grown during summer (April/May to June), followed by maize in rainy season (July to October) and wheat/maize/mustard/chickpea in winter season following recommended package of practices. Besides producing grains, green gram added significant amount of N (30-40 kg/ha) into the soil. Maximum amount of biomass on dry weight basis and highest N input in the soil was from *Sesbania* (126-135 kg N ha^{-1}). Remarkable improvement in the growth and yield of maize, following summer legumes, was also observed in addition to saving of N to the extent of 50–60 kg ha^{-1} with *Sesbania*, and 35–40 kg ha^{-1} with green gram. The succeeding crops grown in winter season also benefited by residual effect of summer legumes and showed N economy of 15–25 kg ha^{-1} after green gram, and 25- 29 kg ha^{-1} after *Sesbania*. There was a significant improvement in soil organic C and nutrient status after six cropping cycles with summer legumes. Results revealed that dual-purpose summer legumes were better options for improving productivity, profitability, N economy and soil fertility of maize based cropping system.

Tillage/Crop Establishment x Nutrient Interactions

Globally, research evidence suggests that adoption of conservation agriculture based management practices under different maize based production systems and ecologies can address the emerging challenges of natural resource degradation, energy, water and labour crises, low nutrient use efficiency and climate change effects. The variable soil environment under contrasting tillage and residue management practices have important bearing on dynamics of nutrients in the soil and influence the nutrient economy and use efficacy. Therefore, understanding nutrient dynamics under contrasting soil management practices is important for managing nutrients in an efficient way. Conservation tillage (CA) practices are increasingly becoming popular in maize systems in India. A nutrient omission study in winter maize (20) under zero- and conventional tillage showed that (**Figure 7**) N, P and K omission plot yields are higher under zero-till situations suggesting higher nutrient availability. Several researchers (23, 39) comparing CT and no-till production systems, suggested that more efficient utilization of fertiliser with no-till production produced higher yields. Pampolino *et al.* (25) also reported similar observations while evaluating NE-Wheat in different tillage options under varied growing environments. This suggests that tillage has a strong influence on nutrient dynamics and their availability to crops, and tillage X nutrient interactions must be addressed while developing nutrient management strategies for maize grown under variable tillage environments.

Nutrient Management Research Gaps

Traditionally, the nutrient management research was primarily focused on developing generalized prescriptions for larger domains and for conventional crop management practices. However, during recent past, conservation agriculture based crop management practices have emerged as one of the potential alternate to conventional tillage based maize production systems. But, still most research advances in nutrient management including SSNM caters to conventional tillage based crop management systems. The contrasting tillage management practices (conventional and conservation agriculture) will have implications on soil moisture regime and nutrient dynamics that in turn will influence nutrient response and economic profitability of nutrient application. Therefore, there is a need to develop prescriptions and application strategies in line with the 4R principles (right source, right rate, right time and right place) for conservation agriculture based maize systems. Therefore, to implement best management practices for plant nutrients at different scales our future nutrient management research in maize systems should focus on the following:

- Crop physiological processes and efficiency under contrasting management practices will be variable that will lead to variable nutrient responses. Basic understanding of such processes will allow designing appropriate nutrient management decision tools/prescriptions.
- Nutrient availability under enhanced moisture availability under conservation agriculture scenarios needs to be understood properly to determine appropriate rate and time of nutrient application.
- Scientific basis of attainable yield targets need to be established under contrasting management practices for tillage and residues in various cropping systems under diverse ecologies (rainfed, irrigated).
- Calibrating sensors for nutrients not only for N but also P, K, Zn, etc.
- Establish relationships for on-the-go remote sensing sensors and satellite remote sensing for SSNM.
- Use of remote sensing and GIS for mapping fertility variability and making nutrient prescriptions at different scales.
- Geo-referencing/mapping of large domains for developing homologous regions for nutrient prescriptions.
- Develop, validate, and bring to scale decision support tools (Nutrient Expert) and farmer friendly simple practices for system based SSNM for small holder precision.
- Develop and deploy regional recommendations that can be distributed through ICT solutions
- Development of appropriate machinery for nutrient application (surface application, drilling, band placement, fertigation) under different management scenarios (no-till with and without surface

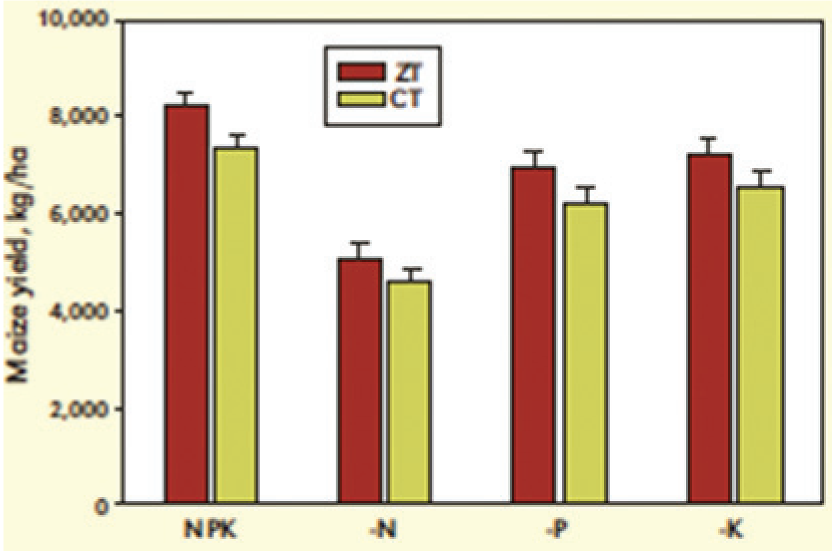

Figure 7 – Average yields of winter maize in omission plot trials under zero-till (ZT) and conventional till (CT) systems

residues, conventional till with and without residue incorporations) is urgently required.

CONCLUSION

In India, maize has traditionally been grown as subsistence crop in unfavourable ecologies and hence there exist large management yield gaps in maize systems under different ecologies. Large proportion of these management yield gaps are contributed by imbalance and inappropriate plant nutrition and multiple nutrient deficiency. However, under the emerging resource constrained and variable climatic conditions, there has been a growing realization for maize to feed the future. Therefore, the technological advancements in maize systems needs twin shifts from subsistence to commercial maize farming and from production oriented to profit oriented sustainable farming. Therefore, defining precise recommendation domains for fertiliser best management practices for plant nutrients in maize systems and their implementation using modern tools, techniques and approaches have to play major role not only for bridging yield gaps but also for improving nutrient use efficiency, economic profitability and reducing losses and to address climate change issues.

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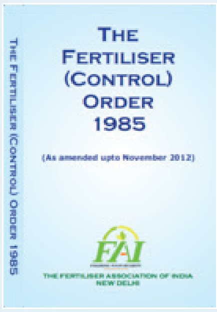

agronomy

Editorial

Towards a Better Understanding of Agronomic Efficiency of Nitrogen: Assessment and Improvement Strategies

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Abstract: Agronomic N-use efficiency is the basis for economic and environmental efficiency, and an effective agro-ecosystem management practice, improving nutrient use efficiency, is a crucial challenge for a more sustainable production of horticultural, industrial and cereal crops. However, discrepancy between theory and practice still exists, coming from large gaps in knowledge on net-N immobilization/mineralization rates in agro-ecosystems, as well as on the effects of indigenous and applied N to crop response. A more thorough understanding of these topics is essential to improve N management in agricultural systems. To this end, the present Special Issue collects research findings dealing with different aspects of agronomic efficiency of N in different agro-ecosystems, and environmental impact derived from fertilization management practices. In particular, the Special Issue contains selected papers, which concern a wide range of topics, including analyzing tools, options of management, calculation equation and modeling approaches.

Keywords: nitrogen use efficiency; cropping systems; nitrogen management; calculation tool; fertilizer source; nitrate leaching; crop N-status; modeling approach

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1. Introduction

Agricultural production would need to increase by 70% (on average) by 2050 to cope with the growth of the world's population [1], which is forecasted to reach about 9.6 billion people [2]. The required crop production increase would be derived from higher yields and enhanced cropping intensity, which, in turn, will raise the demand for agricultural input. However, in this scenario, it is crucial to point out the potential worsening of soil degradation, water resource pollution and atmospheric contamination. Thus, suitable agronomic practices, and particularly fertilization strategies (e.g., organic fertilizers and amendments application), will need to be used.

There is a considerable amount of literature showing that an effective agro-ecosystem management, improving nutrient use efficiency, is a crucial challenge for a more sustainable production of horticultural, industrial and cereal crops. As a matter of fact, as agronomic efficiency (*i.e.*, nutrients recovered within the soil-crop system) improves, economic (farm income is maximized from proper use of nutrient inputs) and environmental efficiency (reduced risk of nutrient losses) will benefit [3].

Nitrogen (N) is a critical element for plant growth, which is also the most complex one considering all of the potential forms and processes involved in its cycle. However, it has been estimated that 50%–70% of the N provided to the soil is lost due to volatilization, runoff, denitrification and leaching [4]. Improving nitrogen use efficiency (NUE) of cropping systems is essential in order to reduce environmental risks, obtaining, at the same time, a productive agriculture. This result can be achieved by reaching a greater plant uptake efficiency from applied N inputs, and reducing the amount of N lost by soil organic and inorganic N pools. Therefore, there is a need to synchronize crop N demand and the N supply, in time and space, throughout the growing season, not for single crops, but also for a complex crop rotation, including cover crops as an integrated system.

As for the N rate, over- or under-application always results in reduced NUE, yield and crop quality. Moreover, it should be considered that potential nitrate leaching from organic N sources (e.g., green manures, compost, digestates) can be equal to or greater than potential losses from inorganic N fertilizer, when the available N supply exceeds crop demand.

The existing discrepancy between theory and practice comes from large gaps in knowledge on net-N immobilization/mineralization rates in agro-ecosystems, as well as on the effects of indigenous and applied N to crops response. A more thorough understanding of these topics is essential for improving N management in agricultural systems, which should be addressed mainly with: (i) proper fertilizer N management strategies (type of input, time, method and amount of application); (ii) slow-release fertilizers and nitrification inhibitor use; and (iii) diversified crop rotation, including cover crops to capture or recover residual N in the soil, after a main crop harvest.

On the whole, better prediction of soil-available N supplies, crop N, and water needs can improve NUE by tailoring fertilization to the specific conditions of sites, thus optimizing crop performance.

2. Special Issue Overview

This Special Issue collects current research findings dealing with different aspects of the understanding of agronomic efficiency of N in the agro-ecosystems and the environmental impact derived from the adoption of fertilization management practices. In particular, the Special Issue contains seven selected papers, which concern a wide range of topics, including analyzing tools, options of management, calculation equation and modeling approaches. Therefore, in this Editorial, we will briefly introduce the papers published in our Special Issue, entitled "Towards a Better Understanding of Agronomic Efficiency of Nitrogen in Different Agro-Ecosystems".

The papers can be broadly organized into two main subjects: (i) nitrogen efficiency assessment and (ii) nitrogen efficiency improvement strategies, which are hereafter outlined.

2.1. Nitrogen Efficiency Assessment

The first paper by Weigh [5] is a Technical Note presenting a calculation tool for analyzing NUE in different varieties of wheat and biomass-willows crops. What is interesting about this study is the potential solution provided for plant mean N content determination during critical crop growth stages, avoiding to perform destructive plant harvests at the exact dates of those stages for all treatments and cultivars. As underlined by the author, a prerequisite for any improvement of NUE of crops grown in different agro-ecosystems is appropriate assessment by using methods that allow for comparisons across crops, varieties, experimental setups and different sites. A feasible application of the tool is the validation of techniques to improve the NUE of different crops.

In Lv *et al.* [6], a methodological tool for a more suitable seasonal fertilization approach is proposed, using a set of calculation equations. Equation application parameters were collected from more than 50 long-term and short-term field tests, being the base of balanced fertilization to properly increase or reduce nutrient rate.

Finally, the paper of Piccini *et al.* [7] deals with assessing nitrate losses in an Italian ryegrass-silage corn crops rotation of a buffalo livestock farm, by using a modeling approach (WinEPIC model) and comparing data trends with NO_3^- concentration, measured into lysimeters. Three scenarios were simulated, with different fertilization rates, showing a beneficial effect on N loss reduction and NUE improvement without any substantial decrease in forage crop yields. Thus, it is suggested that the model proposed by the authors can be used to predict the effect of fertilizing practices.

2.2. Nitrogen Efficiency Improvement Strategies

The first paper of this group, by Nelson *et al.* [8], pointed out that enhanced-efficiency N sources (urea-based fertilizers) can increase wheat profitability. In particular, polymer-coated urea (PCU) or *N*-(*n*-butyl) thiophosphoric triamide (NBPT) treated urea, can increase fertilizer uptake and enhance NUE by reducing N loss. In a four-year field experiment, the authors found that PCU is a viable option for fall application to wheat in poorly-drained soils, whereas, in well-drained soil, fall-applied ammonium nitrate results in greater wheat yields than other N sources. Therefore, the specific pedo-climatic conditions of a site always play a crucial role.

In Agneessens *et al.* [9], two alternative strategies of vegetable crop residues management to reduce nitrate leaching (during winter season) in intensive crop rotations were reviewed: (i) on-field management options and modifications to crop rotations and (ii) removal of crop residues, followed by a useful and profitable application. The conclusion of this complex Review study is that valorization of vegetables crop residues through composting, anaerobic digestion, or ensilage should be promoted, aiming at returning them in a more suitable form to the field, to maintain soil organic matter and nutrient reserves, and also maximizing synchronization between N availability and demand.

The subject of nitrate leaching reduction was also faced by the paper of Herrera *et al.* [10], reporting a study that was conducted by using lysimeters, as in Piccinni *et al.* [7]. The aim was to determine whether three spring wheat genotypes have the potential to minimize nitrate leaching during spring and summer. Unfortunately, the genotypes varied in fertilizer N recovery but not in N losses by leaching, since root growth and N uptake were not well synchronized with nitrate leaching, which occurred before the stage of stem elongation. The paper suggests that the ability to minimize N losses by using spring wheat genotypes differing in N uptake could be site-specific.

Finally, an up to date Review on the different strategies that can be used or developed for increasing NUE in cereals is presented in Herrera *et al.* [11]. This review article also places the focus on the importance of improving NUE using innovative technologies, such as nanofertilizers and endophytic microorganisms. A detailed description of the N sources commonly used in cereals' production is also presented, along with N management practices to optimize source, rate and method of application, and methods used to assess the N status of crops.

3. Conclusions

Agronomic N-use efficiency is the basis for economic and environmental efficiency, and more sustainable agricultural practices are required in farm management for improving crop yield performance and to reduce the environmental risks of farming.

This Special Issue contains different papers, serving as an update on different aspects of knowledge concerning the agronomic efficiency of N in agro-ecosystems, including analyzing tools, options of management, calculation equation and modeling approaches. The aim should be to minimize the discrepancy between theory and practice, coming from large gaps in knowledge on net-N immobilization/mineralization rates in agro-ecosystems, as well as on the effects of indigenous and applied N to crops response.

We are confident that this Special Issue will stimulate further research in the field of agronomic efficiency of N for several agricultural systems.

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Abbreviations

The following abbreviations are used in this manuscript:

N	Nitrogen
NUE	Nitrogen Use Efficiency

PCU	polymer coated urea
NBPT	*N*-(*n*-butyl) thiophosphoric triamide

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- Review Article

GENETIC APPROACHES OF INCREASING NUTRIENT USE EFFICIENCY ESPECIALLY NITROGEN IN CEREAL CROPS – A REVIEW

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Abstract

Nutrient use efficiency determines precisely a certain amount of plant yield in terms of grains or biomass per unit of applied nutrients. Crop plants contain many more elements but for their growth and development basically they require almost sixteen mineral nutrients, among which N, P, K are used in large amount and N covers manifold function for contribution of the yield attributes. In deficiency of any micro- or macro-nutrient and due to few environmental factors plant growth along with its yield are affected adversely. In addition to physiological and breeding strategies genetic progress and implications have characterized this area to some extent. As nutrient efficiency is expressed in several ways, this phenomenon is taken in a wrong sense among the concerned persons and thus, there should be a balance between optimum nutrient use efficiency and optimum crop productivity based on the selected phenotypic characters of crop plants. Nutrient use efficiency is said to be a complex trait. Even its different stages of action are also considered complicated in nature. In such case a single gene can provide huge benefit. This is why modern genetic tools and resources available to the scientists have provided a great possibility for increasing nutrient use efficiency in crop plants. Molecular biology offers possibility for improving the desired characters by introduction of the specific gene(s). Augmentation of nutrient content of crop plants is being caused through genetic engineering. This article makes review and discussion on the genetic approaches in terms of exploited genetic engineering and biotechnological tools for increasing the specific nutrients especially nitrogen in cereal crops.

Key words: Genetic Approach, Nutrient Use, Cereal Crops.

Introduction

Plants nutrients are divided into two groups and that are *macronutrient* (used in large quantities for plant structure) and *micronutrient* (used in small quantities for enzymatic process). Mengel and Kirby (1978) classified plant nutrients into four groups on the basis of their biochemical behaviour and physiological function. All these elements are also classified as cations, anions, metals and non-metals based on their specific functions in the plant body. Plants can absorb inorganic nutrients by themselves and organic form of nutrients undergoes mineralization for up-taking by plants. Also transportation of nutrients and their absorption by roots and translocation for assimilation in plant body happens eventually. Nutrient uptake by plant is a catenizing process. In passive process, ions move from higher to lower concentration and in active process, ions move against a concentration gradient.

Nutrient deficiencies vary among soils and areas, and N and P are the most deficient nutrients in temperate as well as tropical soils. Among the essential nutrients, K is absorbed in maximum amount by the modern improved crop cultivars (Fageria *et al.* 1991), when N is considered as a major limiting factor in plant productivity. Nevertheless, enormous use of N fertilizers in inorganic form for the last few decades showing a detrimental environmental effects. Now it has become essential to reduce N fertilizer pollution, rather than it is strengthening the importance of improving the nitrogen use efficiency of crop plants.

In an edited book by Tandon (1995) it has been said that micronutrients sometimes are of macro importance for producing qualitative high yields. Zinc (Zn) is a good case in point of micronutrient which has greatest importance in plants life. In terms of nutritional constrains Zn's position is next to N and P. Zinc deficiency is

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actually a great problem to crop plant as well as animals including human beings of Indian sub-continent. Iron (Fe) and Boron (B) are also next to zinc in its importance to the living word. Hence, it is very important task now for the scientist to ensure that the requirements of plants for micronutrients can be accounted by participation of all these elements in enzymatic reactions and as constituents of growth hormones. Since the functions of all the sixteen essential elements are character specific, they singly or sometimes by interaction among them form part of molecule, and becomes essential in plants life cycle. For example N is essential for protein and Mg is for chlorophyll in addition to their few other functions. Also the element exerts its effect directly on growth or metabolism and not by some indirect effect such as antagonisms of another element present at a toxic level (Fageria *et al.* 1991).

In developing countries micronutrient deficiencies result many types of health irregularities. A significant number of peoples take three cereals (wheat, rice and maize) as major source of dietary energy, but they are said to be very much poor for Fe nd Zn and even for vitamin A. So it has become purely an important task now to incorporate extra nutrients in food, as a matter of public health policy. Scientist always has targeted higher crop yields but nutrient contents on the contrary have never been considered as prime goal. Practically increase in crop yield as a result of chemical fertilizers (like N, P, K) application has been ultimately provide lower state of micronutrients. Now-a-days micronutrient deficiency is considered as limiting factor for crop yield and thus, to find out the yield potential crop, genetical correcting measure is very much necessary.

Fixen (2006) stated that the value of improving nutrient use efficiency is dependent on the effectiveness in meeting the objectives of nutrient use. Here, objectives are economical optimum nourishment to the crop, minimizing nutrient losses from the field, sustainability of soil quality components etc. However, in order to identify and understand the regulation of genes involved in enhancing nitrogen use efficiency (NUE) proper evaluation of combined genetic and transgenic approaches to improving NUE should be required as component of any crop improvement programme. The benefits of growing NUE efficient crops will not be realized until breeders evaluate N metabolism and nitrogen use efficiency in economically important crop plants (Shrawat and Good 2008).

Scientists have made remarkable improvement to unfold many plant genotypes with increased amount and uses of micronutrients in staple food crops. These are being exploited now-a-days for increasing micronutrient levels and also for eliminating antinutrient substances (phytic acids, tanins etc.) by breeding as well as genetic engineering techniques. Currently many transgenic plants have been obtained with increased amount of Fe, Zn, vitamin A and S-containing amino acids.

Transgenic strategies for nutritional enhancement may offer rapid and more selective way to introduce the desirable traits in crop plants. Molecular biologists are trying to provide tools for completely molecular and physiological analysis of transformed plants, and some of them are being used to increase nutrient content of crop plants, obviously for better quality food. This article makes review and discussion on the genetical and biotechnological tools, exploitable to increase the content of specific nutrients especially nitrogen in specific cereal crops.

Genetic specificity and mineral nutrients

Efficiency of using certain mineral elements for biomass production is considered as one of the important factors and it is highly related to genetic specificity of plants. Due to advancement of different experimental approaches, experiments on varietal specificity of mineral nutrition has been conducted in certain plant species, such as wheat, maize, sugar beet etc. from various methodological aspects. It has now become evident that individual plant species show specific requirements for certain mineral nutrients.

Micronutrient efficiency is genetically controlled, and the physiological and molecular mechanisms of micronutrient efficiency of plants are just beginning to be understand (Khoshgoftarmanesh *et al.* 2010). Large ranges of genotypic variation in response to micronutrients deficiency stress have been reported in different plant species, particularly in cereals (Graham *et al.* 1992, Cakmak *et al.* 1997, 1998). Such large variation is said to be promising for developing the plant genotypes. Graham and Rengal (1993), Bouis (1996) and Graham and Welch (1996) stated that micronutrient efficient genotypes may provide a number of other benefits, such as reduction in the use of fertilizers, improvement in seedling vigour, resistance to pathogens, and enhancement of grain nutritional quality.

Plant nutriomics

Li (1985) stated that a second green revolution is required that does not rely on intensive fertilization; rather it would aim at improving crop yields in soil with reduced fertilizer application. This would be possible if new crop varieties are developed with enhanced adaptation to low fertility soils. This approach may be applicable for both soils with over fertilization in high input areas, and soils of low fertility in low input areas that are deficient in a number of major nutrients such as N, P, K and other essential elements. However, it would be preferable to identify and select specific traits that are directly related to a specific nutrient efficiency (Yan *et al.* 2006). Once clearly identified, these traits could be used for more efficient screening in controlled environments, or tagged with molecular markers and then improved through marker assisted selection or gene transformation. The author also suggested that useful traits of nutrient efficiency may be associated with altered physiological and biochemical pathways in adaptation to nutrient stress. To achieve it, a systemic study is needed to understand genomic, transcriptomic, proteomic and metabolic aspects of nutrient efficiency, which is called as a whole '*plant nutriomics*'.

Plant nutrition studies look at nutrient efficiency mainly at the whole plant level, although useful studies with whole plant can not provide sufficient insight into the genetic nature and its specific modification of the nutritional processes (Yan *et al.* 2006).

Recent studies in molecular biology have provided possible means to tackle the complex plant nutritional problems through genetic approaches, which together with phenotypic analysis can elucidate the functions and interactions of plant nutrients at the molecular, cellular, organ and whole plant levels. This concept of plant nutriomics was presented schematically by Yan *et al.* (2006). However, this concept based on essential mineral nutrients is redrawn as follows (Fig. 1).

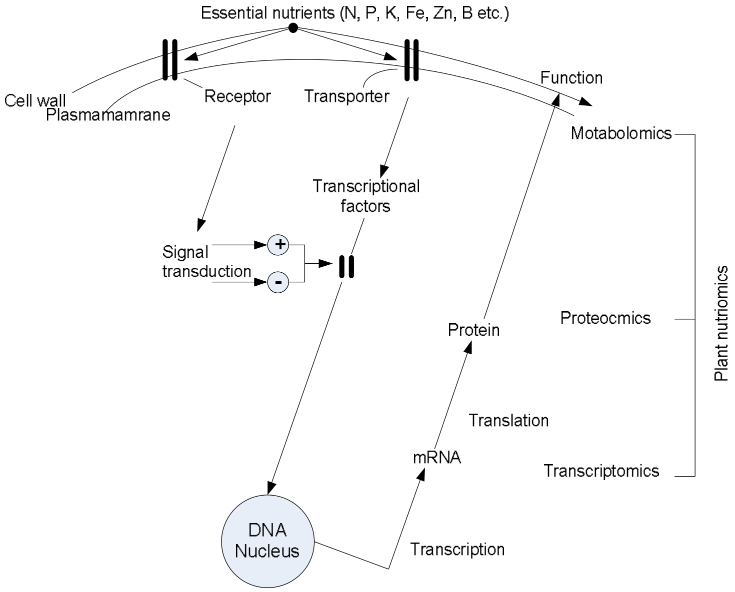

Fig. 1. Line diagram of the basic concepts of plant nutriomics

This line diagram indicates that plant nutriomics is still at a conceptual stage and thus, substantial efforts should be made worldwide for increasing plant nutrition efficiency through genetic and molecular approaches (Yan *et al.* 2006).

Nitrogen use efficiency (NUE)

In case of nitrogen use efficiency (NUE) transgenic overexpression of primary N assimilatory genes in model plants did not yield any significant improvement (Lightfoot 2009, Pathak *et al.* 2008), although physiological experiments on wheat indicated that the expression level of primary N assimilatory enzymes might matter for NUE in some cases (Pathak *et al.* 2011). They stated further, marker studies indicated that genes of secondary N metabolism could be more critical for NUE than the genes of primary N assimilation, especially in cereals which mobilized internal N pools from senescing leaves during grain filling. Apart from genes belonging to the N assimilatory pathway, various other candidates are being identified through screening of different varieties for their NUE under limiting conditions (Chardon *et al.* 2010).

Biotechnological interventions to improve nitrogen use efficiency (NUE) have largely revolved around manipulation and overexpression of many crucial candidate genes apart from using knockout mutations to asses its effect on biomass and plant N status and overall yield (Good *et al.*2004; Masclaux-Daubresse *et al.* 2010, Pathak *et al.* 2008). In case of cereals, the total grain biomass or grain N content would be most suitable indicators of NUE, rather than any other organ (Pathak *et al.* 2011). The same authors suggested that areas where NUE can be targeted is to improve the distribution of N between leaves and stem and roots, better photo-synthetic rate/unit leaf N, reduced leaf senescence, transgenic developing C_4 options for rice and wheat.

Pathak *et al.* (2011) stated that there are two types of nitrate uptake system and these are (i) low affinity transport system encoded by NRTI gene family and (ii) high affinity transport system encoded by NRT2 gene family. In addition, a number of ammonium transporters and putative amino acid transporters have also been identified in *Arabidopsis.* Though the various nitrite and ammonium transports in plants are very well characterized and their differential regulation mechanisms are well known, overexpression studies involving their genes have not been conclusive. Pathak *et al.* (2011) have presented several attempts at transgenic manipulation of the enzymes of primary and secondary assimilation, from there the plant transgenics and the observed phenotypes of cereal crops only are mentioned below (Table 1).

Table 1. Plant transgenics and observed phenotypes in cereal crops.

Gene product	**Gene source**	**Promoter**	**Target plant**	**Phenotypes**
GS2-Chloroplastic glutamine synthetase	*O. Sativa*	CaMV35S	*O. Sativa*	Enhanced photorespiration, salt tolerance
GS1-Cystolic glutamin synthetase	*P. vulgaris*	Rubisco	*T. aestivum*	Entranced capacity to accumulate nitrogen
NADH-GOGAT-NADH dependent glutamate synthetase	*O. sativa*	*O. sativa*	*O. sativa*	Enhanced grain filling, increased grain weight
GDH-glutamate dehydrogenase	*E. coli*	OsUBI	*Z. mays*	Increased N assimilation, herbicide tolerance, biomass, grain aa content
GDH-glutamate dehydrogenase	*C. sorokiana*	CaMV35S	*T. aestivum*	Schmidt and Miller, 2009 (patent No. 627, 886)
GDH-glutamate dehydrogenase	*C. sorokiana*	CaMV35S	*Z. mays*	Schmidt and Miller, 2009 (Patent No. 627, 886)
OsENOD93-1	*nodulin gene*	35sC4PDK	*O. sativa*	Increased shoot mass and seed yield.

Source: Pathak *et al.*, 2011

Remobilization of N is considered as one of the important step in improving NUE in plants (Mascluaux-Daubreasse *et al.* 2010). In case of cereals, grains main source of N is found to be N remobilized from the vegetative parts. It accounts for 60-92% of the N accumulated in grain and the remobilization rate depends upon availability of N and remobilization efficiency. However, it is know that environmental factors along with genotype affect the phenomenon and eventually genes take part for translocation and remobilization attractive targets for improving nitrogen use efficiency.

There are several reports of transgenic overexpression of AS genes that are actively involved in remobilization and translocation of amino acids resulting in enhanced seed protein content and total protein content (Pathak *et al.* 2011). Molecular manipulation of asparagine synthetase (encoded by Aln 1 gene) has been attempted recently along the reports of genetically engineered plants overexpressing alanine amino transferase (Good *et al.* 2007). As translocation and remobilization of N discussed by Pathak *et al.* (2011), genetically engineered rice (*Oryza sativa*) was developed by introducing barley alanine aminotransferase complementary DNA (cDNA) driven by tissue specific OsAnt1 promoter (Shrawat *et al.* 2008). These plants showed improved biomass and grain yield along with significant change in key metabolites and nitrate content confirming increased NUE. However, Beatty *et al.* (2009) reported the involvement of candidate gene through root and shoot transcriptome analysis in engineered rice over expressing alanine amino transferase (AlaAT) under the control of tissue specific promoter showing a strong NUE phenotype. The role of GDH in N remobilizatin is said to be controversial, but transgenic plants over expressing gdhA gene were shown to have improved amino acid content and thereby higher yields in maize and wheat (Lightfoot 2009).

The genes act for N metabolism and nitrate signaling are found to be tightly regulated by sugar signaling mechanism. Pathak *et al.* (2011) said that a coordination between N and C metabolism is required at the amino acid synthesis level due to requirement of carbon skeletons for their synthesis, SnRK1, a principal regulator in carbon signaling, is know to be linked to N and amino acid metabolism. Nitrate is a potential signal that affects N and C metabolism as well as organ growth and development in plants.

However, overexpression of cystolic glutamine synthetase GS2 genes was performed in *Oryza sativa* using CaMV35S promoter (Hoshida *et al.* 2000). More recently, overexpressing a GS1 isoenzyme of maize in maize under the control of the CsVMV promoter increased kernel number and kernel size, thus increasing yield by 30% (Masclaux Daubresse *et al.* 2010). Although overexpression of GOGAT genes has been rare, Yamaya *et al.* (2002) reported a spectacular effect of the overexpression of NADH-GOGAT under the control of its own promoter in rice. Transgenic plants showed an increase in grain weight. They concluded the studies saying that overexpression of GS or GOGAT genes can improve biomass and grain yields depending on which gene allele and which promoter are used.

For nitrogen storage under anaerobic stress alanine performs the function as a major amino acid. Overexpression of barley alanine aminotransferase under the control of root promoters in canola and rice had interesting effects, considerably increasing plant biomass, seed yield, NUE and shoot nitrogen concentration when plants were grown at low nitrate supply (Good *et al.* 2007; Shrawat *et al.* 2008). Hibbered *et al.* (2008) suggested that engineering C_4 rice or C_4 wheat would be a good way to improve NUE.

Manipulation of nitrogen remobilization

Nitrogen remobilization is a complex metabolic process, but it is important for plant productivity because through this mechanism organic nitrogen is recycled to developing leaves and storage organs (Masclaux *et al.* 2001). Therefore, grain yield of cereals does not depend only on uptake of nitrate before flowering, depends also on the remobilization of leaf N during the maturation of seed. About 80% of the total nitrogen in panicle of rice arises due to remobilization through phloem from senescing organs (Tabuchi *et al.* 2007).Consequently, efforts have been made for identifying genes encoding proteins, which are activated specifically during remobilization of nitrogen, carbon and minerals at the time of leaf senescence (Gallais and Hirel 2004). Brugiere *et al.* (2000) suggested that cytosolic GS (GS1) reassimilates amonium released from protein hydrolysis. GS1 facilitates synthesis of Gln, which is the major form of reduced N in phloem sap, and NADH-GOGAT1 is important for developing sink organs for Gln remobilization in rice (Andrews *et al.* 2004). This is why, in case of senescing organs of plants synthesis of Gln is said to be an important step for recycling of nitrogen.

Martin *et al.* (2006) investigated the roles of two genes encoding cytosolic maize GS1 (gln-3 and gln-4) by examining the impact of knockout mutations on kernel yield and by overexpressing gln1-3 in maize. They found gln1-4 and gln1-3 double mutants to exhibit reduced kernel size and kernel number, without reduction in shoot biomass production at maturity. Hirel *et al.* (2007) reported that transgeneic maize plants overexpressing gln1-3 gene produced greater kernel numbers under both high and low nitrogen conditions compared to that of wild type. These findings in maize suggest that GS1 plays important role for kernel yield under both low and high nitrogen fertilization.

In case of rice GS1 knockout mutants made by inserting retrotransposon Tso17 into exon-8 or exon-10 of Osgs 1;1 exhibit a remarkable reduction both in growth and grain filling, grown under normal N fertilization. But reintroduction of Osgs 1:1 cDNA under the control of its own promoter into the mutants complement successfully the slow growth of the plants. This study indicates that GS1;1 is important for normal growth and grain filing in rice, and GS1; 2 and GS1;3 are not able to compensate the function of GS1;1 (Tabuchi *et al.* 2005, 2007).

Microarry analysis

It is a technology for measuring transcriptome expression analysis and it has been applied to some important cereal crops to examine the genetic response. Lian *et al.* (2006) measured the effect of low nitrogen stress on rice by reducing the applied ammonium nitrate for 2 hours. They found over 400 overexpressed sequence tags (ESTs) to be regulated differentially in rice roots, and nitrogen uptake and assimilation transcript were found to be unaffected. Transcriptome changes between nitrate-fed ammonium-fed rice were detected by Zhu *et al.* (2006). They found 198 differentially expressed genes (DEGs) involved in signal transduction, stress response, transcriptional regulation and metabolism. Lu *et al.* (2005) made an analysis on the gene expression changes in wheat, applying both organic and inorganic nitrogen separately. They found organic nitrogen treatment to show the largest number of up-regulated nitrogen uptake and assimilation genes, including AlaAT. On the contrary, they found low expression of nitrogen metabolism genes in case of low inorganic nitrogen treatments.

Beatty *et al.* (2009) stated that there have been relatively few microarry studies measuring the effect of a transgene on the plant transcriptome and most of the reports have analysed overexpressed regulatory proteins. Effect of the overexpression of the *Zea mays* transcriptional factor DREB2A on the *Zea mays* transcriptome was measured by Maruyama *et al.* (2004). They reported 51 genes to be expressed differentially in transgenic relative to wild type plants under non-stressed conditions.

Hirose *et al.* (2007) overexpressed the response regulator OsRR6 (negatively regulates cytokinin signaling) in rice and found 667 and 641 transcripts up-regulated in roots and shoots, respectively. They also found 591 and 962 transcripts down regulated in roots and shoots, respectively. In both the cases they used a two-fold cut-off. Baudo *et al.* (2006) overexpressed the storage protein glutenin in wheat using a 1.5 fold expression difference cut-off. They found five differentially expressed transcripts from a 9426 EST cDNA wheat microarray. Their findings indicate very little effect of the overexpressed storage protein gene on the wheat transcriptome.

Transgenic efforts to nutritional enhancement

Transgenic efforts may enhance the nutritional value of crops. This approach can make the way very much rapid to introduce desirable traits into elite varieties. Zhu *et al.* (2007) stated that transgenic approaches differ from other approaches in that novel genetic information is introduced directly into the plants genome. The chosen approach depends predominantly on whether the nutritional compound is synthesized *de novo* by the plant or obtained from the environment. They further stated that organic molecules, such as amino acids, fatty acids and vitamins are synthesized by plant. Capell and Christou (2004) said that increasing the nutritional value requires some form of metaboling engineering with the aim of increasing the amount of these desirable compounds, decreasing the amount of a competitive compound or even extending the existing metabolic pathway to generate a novel product.

Modern genetic and molecular technologies provide a number of tools that can be utilized for the development of staple foods with a higher iron and zinc content and improved bioavailability of these minerals (Holm *et al.* 2002). Their research summarizes current strategies aimed at increasing the iron-sequestering capacity of the endosperm and improving mineral bioavailability via in planta synthesis of microbial phytases. They have presented a case study of wheat and have discussed the future strategies addressing the importance of phytase thermostability. Their work was to engineering wheat for constitutive and endosperm-specific expression of an *A. niger* phytase (Brinch-Pedersen *et al.*, 2000). Their design was comprised of the strong constitutive promoter from the maize ubiquitin 1 gene and the *A. niger Phy*A gene. To ensure targeting to the cell wall, Holm *et al.* (2002) introduced a signal sequence from barley *a*-amylase upstream of the *Phy*A gene (Ubi-Sp-phyA). The constructs were then introduced into wheat mature embryos by particle bombardment and transgenic regenerable cell lines selected using the bar-Bialaphos selection technique. In their study, western immunoblotting with polyclonal antibodies raised against the *Aspergillus* phytase indicated that a phytase of the expected molecular weight had been synthesized and further that the protein as expected was glycosylated. The *A. niger* phytase contains 10 Asn-linked consensus sites (Asn-

Xaa-Ser/Thr) and the mature fungal enzyme is known to be secreted, glycosylated protein (Van Hartingveldt *et al.*, 1993). However, Holm *et al.* (2002) stated in their case study that at the early and mid stages of grain filling, the heterologous phytase was primarily synthesized in one or more tissues of the pericarp, seed coat and aleurone, whereas the endosperm was the primary site for phytase synthesis toward the end of grain filling. Their study on progeny analyses revealed that the transgenic trait was transferred to the next generation and that there was an upto fourfold phytase activity than measured in wild type seeds.

However, in recent transformation studies, endosperm specific expression of soybean (Goto *et al.* 1999) or *Phaseolus vulgaris* (Lucca *et al.* 2001b) ferritin gene in rice resulted in an upto threefold increase or doubling, respectively, of the iron content of the seed. This implies that the low iron concentration in the seed may not result from low iron availability for transport, but rather from a lack of sequestering capacity in the seed (Holm *et al.* 2002). On the other hand, Drakakaki *et al.* (2000) stated that when the soybean ferritin gene expression was driven by a so-called constructive promoter to increase ferritin synthesis throughout the plant, there was only an increase in the iron content of the vegetative parts but not in the seed.

In developing countries, the most widely used cereal crops are wheat and rice. In these crops, very small fraction (Wheat 20% and Rice 5%) of iron (Grusak and Della Penne 1999, Grusak *et al.* 1999, Miller *et al.* 1993) is transported from the senescing leaves to the grain. In contrast >70% of zinc is mobilized (Grusak *et al.* 1999). In cereals the two minerals are almost exclusively stored in the husk, the aleurone and the embryo and therefore, large proportions of minerals are lost during milling and polishing (Welsh and Graham 1999). This implies that the full potential of the genotype determined increments in iron and zinc content is not related for improving human nutrition and in this context, the mechanisms underlying iron and zinc transport and deposition in different tissues of grain are of particular importance (Holm *et al.* 2002), from transgenic point of view.

However, in genetic studies many sorts of drawbacks are being alleviated by using molecular markers associated with the traits of interest. Molecular marker genotype can eliminate the undesirable trait and important for selection of complex traits where multiple genes are involved. Marker assisted selection for complex trait like yield with essential nutrients may be an essential tool in producing new varieties.

Genetic engineering

Substantial genetic variations in different food crops for micronutrient and vitamin A are being exploited now-a-days to increase the micronutrient levels and also for eliminating antinutrient chemicals (phytic acid, tannins) by conventional breeding techniques as well as genetic engineering techniques. So in future priority should be given for developing plant genotypes with enhanced amount of micronutrient through genetic approaches. Genetic engineering techniques can be used to create novel cultivars with desired properties (Lonnerdal 2002, 2003).

Examples of this type of approaches are the insertion of novel genes, enhancement of the expression of genes, present but at low level, and depression of the expression of genes or disruption of the pathways involved in the synthesis of inhibitors of nutrient element absorption. Two novel iron-binding proteins have been incorporated into rice (Lonnerdal 2003). Compared to other cereal crop plants rice proteins have low allergenicity and this crop contains no toxic compound. Insertion of a gene for a protein that can bind more metal ions per molecule is needed to achieve a trace element content that is high enough to meet the iron requirement. Ferritin is that type of protein, whose each molecule can bind as many as 4500 atoms of iron (Theil *et al.* 1997). The gene for soybean ferritin has been inserted to rice (Goto *et al.* 1999). These experiments seem to have been done on a smaller scale, but the iron content in a few transformants is 2-3 fold than that of wild type (Lonnerdal 2003). A research group has inserted the ferritin gene from *Phaseolus vulgaris* into rice (Lucca *et al.* 2001a).

Genetic engineering allows incorporation of gene from unrelated organisms to plants through sophisticated and artificial techniques. It leads to direct access and manipulation of genetic information contained in DNA and also leads to create synthetic genes. This technology reduces time taken to obtain the improved cultivar. A flow chart of *Agrobacterium* mediated transformation of cereal crops for a specific gene is show below in Fig. 2.

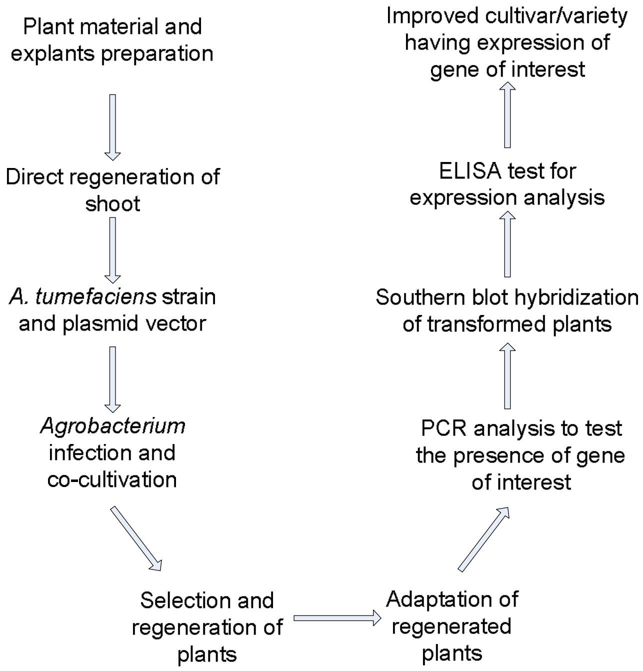

Fig. 2. *Agrobacterium* mediated transformation of cereal crops for a specific gene.

Genetic engineering techniques are only used when other technique are exhausted and when (i) the trait to be introduced is not present in the crop; (ii) the trait is so difficult, not possible to improve it by conventional breeding methods; and (iii) through conventional breeding method it will take a very long time to introduce the traits.

This technology aims in increasing plant productivity. It has been possible by molecular biologists to identify gene(s) whose products participate in diverse metabolic pathways. It is most applicable to compounds of nutritional importance. It may be called nutritional genomics and the great example of this phenomenon is modification of genes, which resulted synthesis of vitamin E in plants.

Recombinant DNA technology

Recombinant DNA technology allows the modification of DNA by the enzyme like endonucleases, lipases and polymerases and these are used by scientists to isolate, characterize and modify the genes, and thereby transferring them into the same or different organism. Hence, the development of transgenic plants involves gene identification and modification of the target gene for expression in crop plants. However, transfer of this modified gene into the plant cells is done by *in vitro* techniques or natural methods. The *in vitro* technique makes the plants containing a high copy number of rearranged or catenated transgenes. This methodology has been found to be simple and efficient in case of monocotyledonous plants, including cereals.

For example, recombinant DNA technology was used to improve the provitamin A content of rice grain endosperm in Golden rice (Ye *et al.* 2000). Goto *et al.* (1999) transformed rice plants using a phytoferritin (Protein storage from of Fe in plants) gene form soybean and rice endosperm promoter gene to enrich Fe in the rice grain endosperm. Lucca *et al.* (2001a) also enriched Fe in rice grain endosperm using the phytoferritin gene from pea plants.

Amino acids and transgenes

Most crops are deficient in certain essential amino acids and that can not be synthesized *de novo* by humans (Zhu *et al.* 2007). Cereal grains such as rice and wheat are found to be deficient in lysine and threonine, in this case, a simple approach may be the expression of recombinant storage proteins with desirable amino acid profiles. As an example of this approach include the expression of pea legumin, which has a high lysine content in rice and wheat grains (Singh *et al.* 1997, Stoger *et al.* 2001).

Geneticists have been attempting to enhance the lysine content in cereals, but have met with unsatisfactory outcomes. It is because of underirable traits, which are often associated with the essential aminoacids, e.g. high lysine content. Zaman and Kabir (2008) made a biochemical analysis of five tip sterile (undesirable trait) varieties of hexaploid wheat. Only four amino acids such as valine, glutamine, glycine and lysine could be identified by them. Proline and aspergine which play important role for fertility of anthers could not be detected in their study. However, high proline content increases in fertile anthers prior to anthesis in contrast to low proline in male sterile anthers. In such case biotechnological progress may allow the use of transgenic approach to increase the content of a specific essential amino acid in a target plant.

Lactoferrin and ferritin are also capable of facilitating uptake of trace element. Various dietary ligands can enhance trace element absorption. One example of this is amino acids, which are largely released from proteins during digestion. Cysteine or cysteine rich peptides are shown to have a positive effect on iron absorption (Layrisse *et al.* 1984, Tylor *et al.* 1986) and histidine is shown to facilitate zinc absorption (Lonnerdal 2000). Organic acids such as fumarate, citrate and succinate can also enhance trace element absorption.

Engineered genes are being used by scientists in tropical indica rice using nonantibiotic selectable marker *pmi* (phosphomenose isomerase) gene (Datta *et al.* 2003). Moreover, iron has been enhanced in indica riece by introducing ferritin gene driven by the endosperm specific promoter (Vasconcelos *et al.* 2003). Datta (2004) stated that it is possible to improve the amino acids (such as lysime) in rice. As it has been shown by pyrimiding transgenes (Xa21, Bt, PR-protein genes) functioning in a homozygous single elite cltivar (Datta *et al.* 2002), it might be possible to incorporate the genes for β-carotene and enhancement of iron and protein in a single rice variety to achieve the dream rice for those who need it most. Datta (2004) stated that nutritions rice with iron and β-carotene in polished seeds has been developed with genetic engineering technology. He further stated that it is now possible to use Bt or Xa21 rice in farmers field with a reduced use of pesticide, and policymakers should look into the potential use of biotechnology, provide access to the intellectual property rights (IPR), and make improved rice seeds available free of IPR for resource-poor farmers.

Tansgenic plants are capable of transmitting the incorporated gene to the next generation. Transgenic strategies have been concentrated for increasing the mineral content, particularly iron (Fe) and zinc (Zn) in crop plants. Two distinct approaches are used and these are (i) increasing the efficiency of uptake and transport to harvestable tissues and (ii) increasing the amount of bioavailable mineral accumulating in plant (White and Broadley 2005). Combination of multiple nutritional improvements into cereal crops without disrupting endogenous metabolic pathways should be achieved in such a way which can ensure transgene and expression stability from generation to generation. This would be achieved through direct integration of multiple genes into a single, permissive transgenic locus (Altpeter *et al.* 2005).

Transgenic approaches have both advantages and disadvantages. The advantages are (i) rapid progress, (ii) unconstrained by gene pool, (iii) targeted expression in edible organs, and (iv) applicable directly to the elite cultivars. The disadvantages are (i) regulatory landscape, (ii) political and socio-economic issues relevant to transgenic plants, and (iii) possible intellectual property constraints. Compared to that of mineral fertilization and conventional breeding/mutagenesis transgenic strategies could help more to alleviate malnutrition of both crop plants and human beings.

Conclusion

Genetically modifying plants for increasing 'nutrient use' efficiency is a complementary approach. By increasing the nutritional element of cereal crops and/or enhancing the bioavailability of the essential macro- and microelements in the staple food crops, nutritional necessity in the developing countries may be improved. Genetic modification can be achieved by two different approaches and these are (i) conventional breeding and (ii) genetic engineering. The second one is the most suitable biotechnological tool for increasing the 'nutrient use' efficiency. This can be achieved by introducing the genes which code for nutrient element binding proteins, over expression of storage protein and/or increased expression of proteins and responsible for nutrient elements uptake into the plants. Finally it can be said that significant advances will be made in near future in the developing countries.

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