

SPECIAL SECTION: TRANSGENIC CROPS

Breeding objectives and requirements for producing transgenics for major field crops of India

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To identify crop improvement objectives in twelve important field crops (rice, wheat, maize, sorghum, pearl-millet, pigeonpea, chickpea, mungbean, cotton, potato, mustard and soybean) that are grown extensively in India, we conducted a survey amongst plant breeders, pathologists, entomologists and agronomists specializing in each of these identified crops. A questionnaire was sent to around fifteen scientists actively involved with each crop with the following queries: (1) Identification of problems with the crop at the regional level in terms of priority, (2) Identification of problems with the crop at the national level in terms of priority, (3) Which are the most extensively grown cultivars of the crop at the regional and at the national levels?, (4) What steps could be taken to raise the yield of the crop (heterosis breeding, pure-line breeding)?, Do you know of combiners that would give high heterosis in the crop?, (5) Do you know of germplasm sources that could be used for meeting some of the breeding objectives?, (6) What is your assessment of need for transgenics (a) for nutritional enhancement, (b) for resistance to biotic stresses, (c) for resistance to abiotic stresses, (d) for herbicide resistance and (e) for value addition?

A list of pests and pathogens known to affect each of the above crops as given in *Handbook of Agriculture* (Indian Council of Agricultural Research, 1997) was also sent to the specialists for help in answering question 2. The five most important problems identified by the specialists in response to the questions 1 and 2 were given scores. The foremost important problem identified was given a score of 5 followed by scores of 4, 3, 2 and 1 for problems identified in descending order of importance. For each of the breeding objectives identified by the respondents, a total score was calculated. Cumulative score for each objective was normalized to a percentage score. In Figure 1, first five problems identified for a crop are given with their scores in top five boxes and all the other problems identified by the respondents are put with a normalized cumulative score in the lowermost box (for all crops except pearl millet). The breeding objectives

identified in relation to regional needs were compared with the objectives identified at the national level and the findings are highlighted in this article. Figure 2 shows the area under cultivation and the production levels of different crops that have been dealt with in this study (except mungbean). This information is presented to highlight changes in production, area under cultivation and yield of the crops over the years.

Rice (*Oryza sativa*)

Rice is the most extensively grown crop of India and is grown in almost all parts of the country. This crop is grown under diverse agro-ecological conditions as irrigated rice, upland rice, lowland rice and deepwater rice. Objectives related to breeding for resistance to various fungal and bacterial diseases and insect pests were identified as the top priorities for rice. Rice blast (*Piricularia oryzae*), sheath blight (*Rhizoctonia solani*) and bacterial blight (*Xanthomonas oryzae*) topped the list of priorities (Figure 1 a). Two insect pests namely, stem borer (*Scirpophaga* sp., *Chilo* sp., *Sesamia inferens*) and brown plant hopper (*Nilaparvata lugens*) cause extensive yield losses in rice and were identified as problems that need attention (Figure 1 a). The respondents in general felt that germplasm for resistance to blast, bacterial leaf blight, tungro virus, stem borer, brown plant hopper, gall midge and whiteback plant hopper is available. However, resistance sources for stem borer, leaf folder, sheath blight and sheath rot were thought to be inadequate. Therefore, the development of transgenics for resistance to the last four biotic stresses needs to be given the highest priority.

Breeding for higher yield through exploitation of heterosis was also identified as a major challenge. For heterosis breeding in rice, several good combiners were identified by the respondents. However, it appears that more extensive search for parental lines with high combining ability is necessary for full exploitation of heterosis breeding. In comparison to all other crops that are included in this survey, rice scientists made most extensive suggestions for the development of transgenics. The following objectives were considered to be worth pursuing

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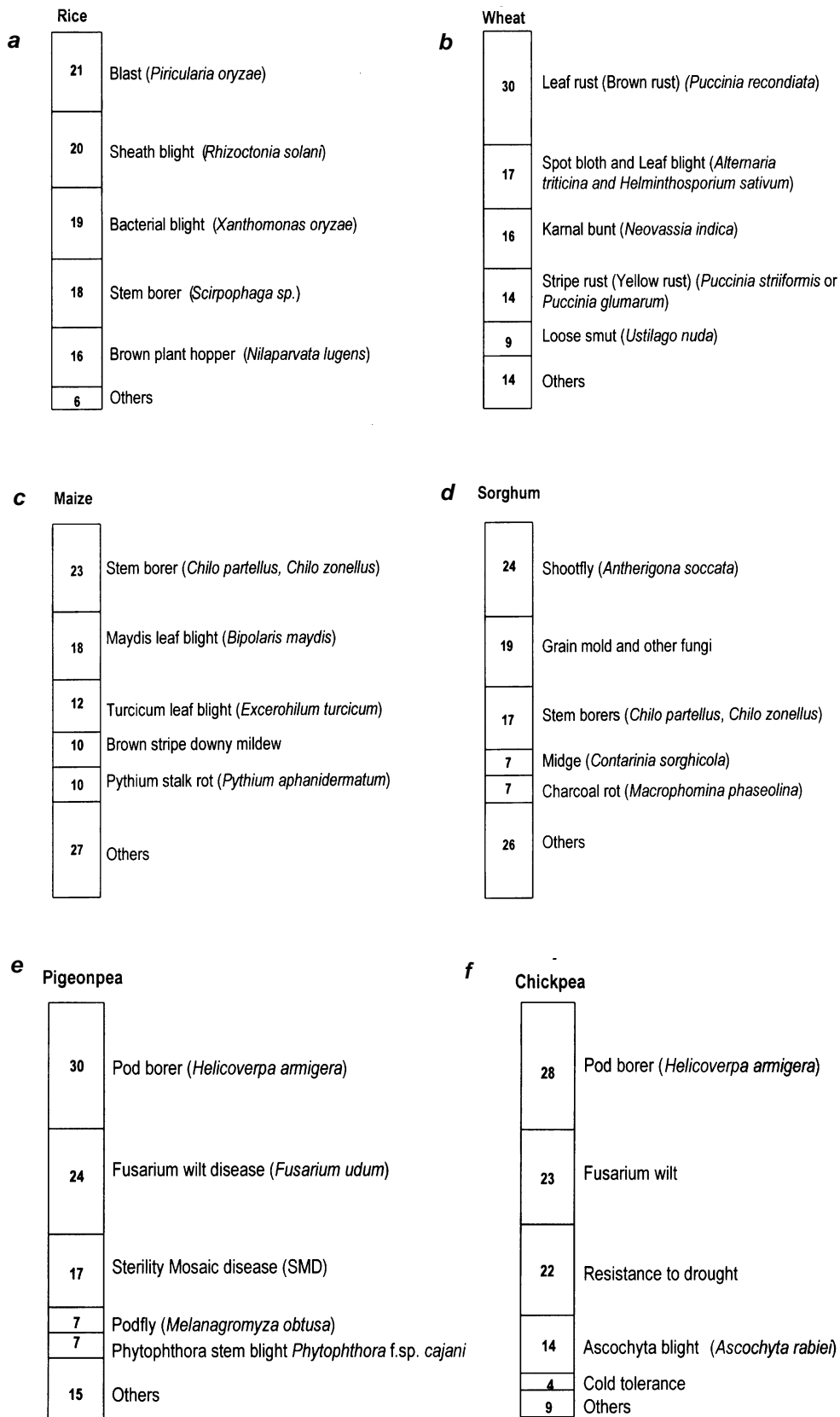


Figure 1 a-f.

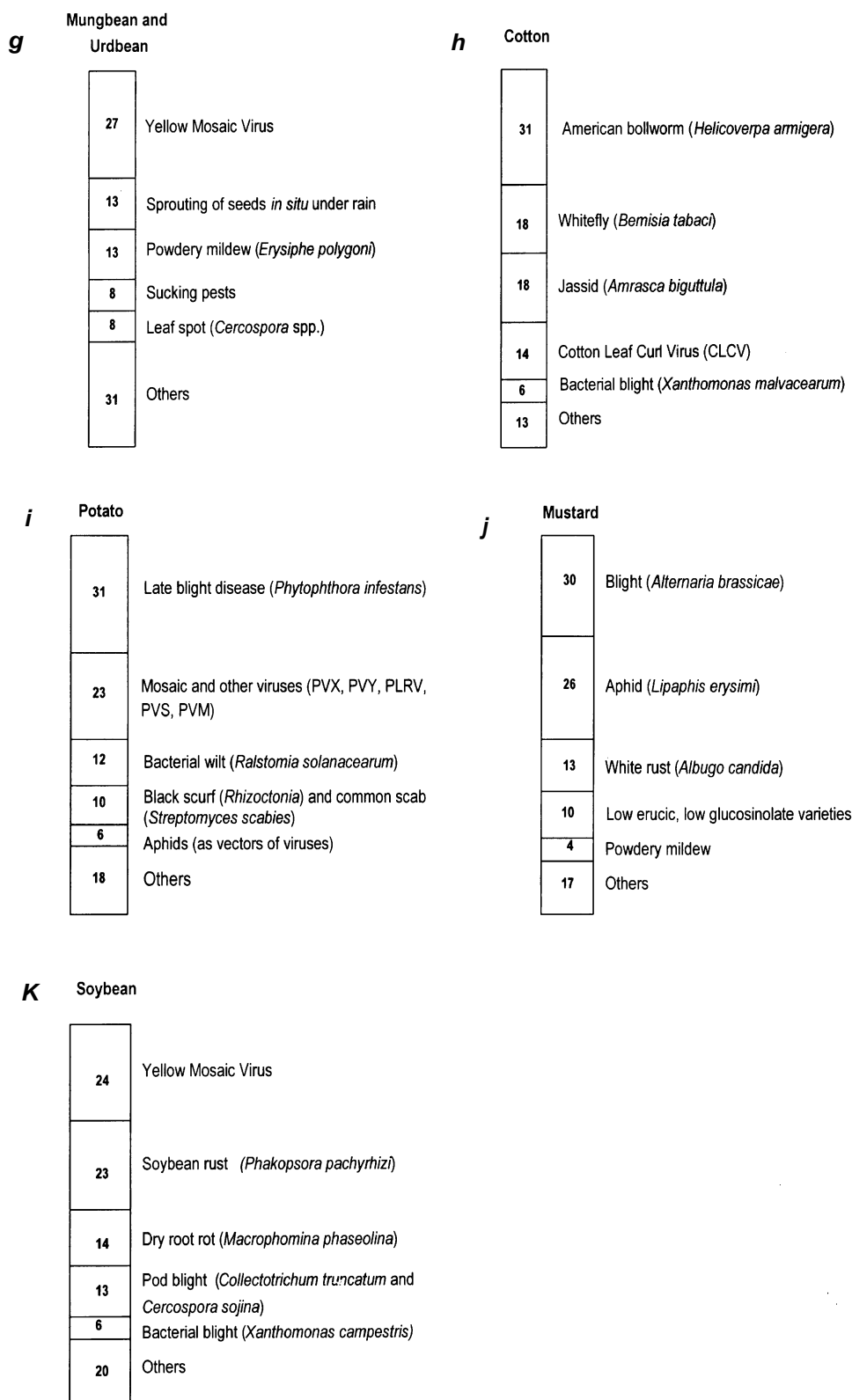


Figure 1. Normalized scores of major constraints that were identified from the survey conducted in this study. The top five problems in each crop are represented with their respective normalized scores. All other problems with a normalized cumulative score are shown in a separate box at the bottom of the histogram column for each crop. The details of problems shown in the 'Others' box are as follows: rice – sheath rot, tungro virus, etc.; wheat – stem rust, powdery mildew, earcockle, hill bunt, flag smut, etc.; maize – bacterial stalk, head smut, charcoal rot, banded leaf, sheath blight, water logging, weeds, etc.; sorghum – anthracnose, smuts, downy mildew, rust, etc.; pigeonpea – water logging, resistance to drought, etc.; chickpea – *Botrytis* grey mould, bruchids on stored grain, etc.; mungbean – storage grain, bruchids, resistance to drought, etc.; cotton – pink bollworm, spotted bollworm, thrips, wilt, etc.; potato – tuber moth, brown rot, nematodes, etc.; mustard-downy mildew, non-availability of hybrids; soybean – *Perenospora* sp., stem borer, green semi-looper, etc.

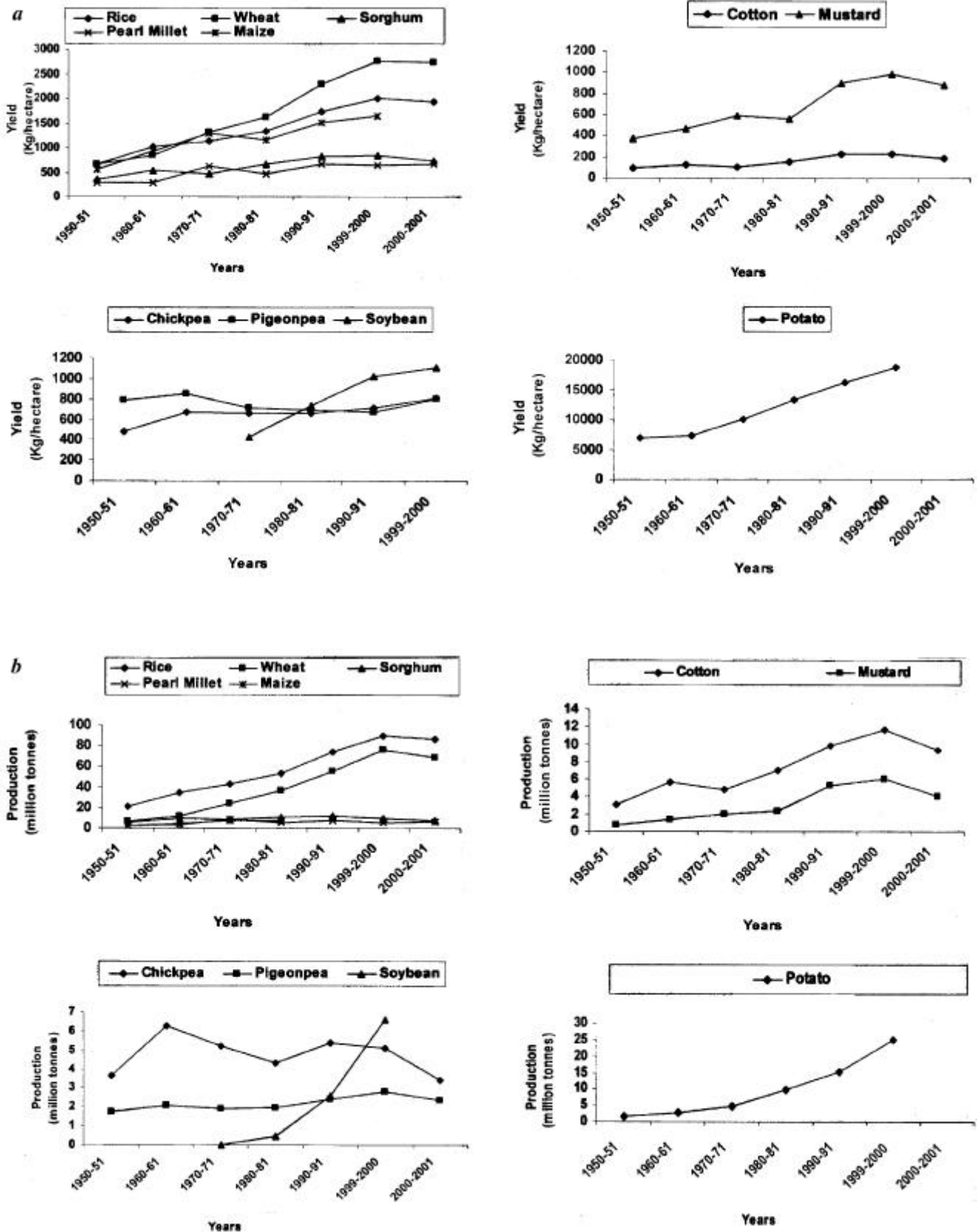


Figure 2 a-b.

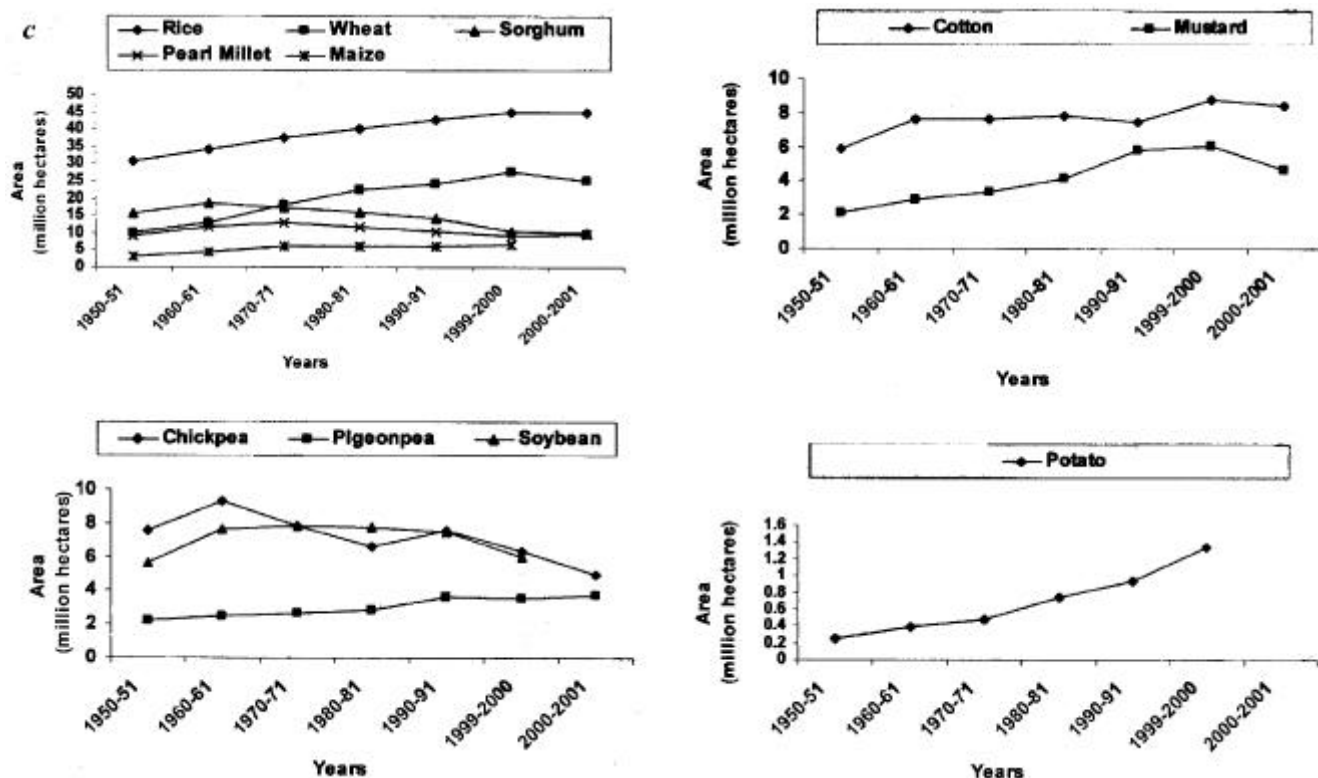


Figure 2. Current production levels and area under cultivation of twelve selected crops in India. *a*, Yield (kg/hectares); *b*, Production (million tonnes) and *c*, Area (million hectares). This figure is based on information available at web site www.agricoop.nic.in.

in this regard: (1) Development of rice with *b*-carotene pathway and higher iron content. (2) Transgenics for resistance to diseases and insect pests. (3) Transgenics for resistance to flooding and drought tolerance, the latter being particularly important at the grain filling stage. Cold tolerance at maturity in rainy season crop and cold tolerance in general for 'boro rice' were considered important. (4) Herbicide resistance was proposed to be particularly important for upland rice and for allowing direct seeding to replace the practice of large-scale transplantation. (5) Quality improvement by incorporation of characters related to aroma and grain length were mentioned, although it is not clear from the survey whether this needs to be accomplished through the development of transgenics or whether it can be achieved by conventional breeding.

In short, biotic stresses take the highest priority in rice breeding followed by abiotic stresses like drought and flooding. For developing resistant rice varieties for both biotic and abiotic stresses, this survey emphasized the use of transgenic technologies.

Wheat (*Triticum aestivum*)

Breeding for resistance to fungal diseases appeared as the most important overall objective in present-day wheat breeding programmes. Leaf rust (caused by *Puccinia recondita*), leaf blight (caused by *Alternaria tritici* and

Helminthosporium sativum), Karnal bunt (caused by *Neovossia indica*), stripe rust (caused by *Puccinia gluamarum*) and loose smut (caused by *Ustilago nuda cv. tritici*) were identified as the relevant problems (Figure 1 *b*). Diseases such as stem rust (*Puccinia graminis*), powdery mildew (*Erysiphae graminis*), earcockle (*Anguina tritici*), hill bunt (*Tilletia foetida* and *T. caries*) and flag smut (*Urocystis tritici*) were also counted. The severity of different diseases varied in different wheat-growing regions of the country: in the most extensively cultivated areas of the North-West, leaf and stripe rusts, foliar blight and Karnal bunt emerged as the major diseases; stem rust (though some specialists felt that this is no more a problem), leaf rust and loose smut emerged predominant in the South and Central peninsular regions; hill bunt and stripe rust were considered common in hills of North India; powdery mildew appeared prevalent in Northern sub-mountainous regions and North-Eastern region appeared to have leaf rust, loose smut and foliar blight. The respondents felt that enough germplasm was available in both cultivated varieties and related wild species to breed for resistance to major diseases. However, not many sources of resistance to leaf blight were enlisted. Karnal bunt appeared an important disease and development of varieties for resistance to Karnal bunt by conventional as well as transgenic means emerged to be important considering the possibility of using surplus wheat for exports.

Pure line breeding has been, by and large, successful in wheat. Breeding for disease resistance in wheat is a major success story in India. However, since the introduction of dwarf wheat varieties in 1960s, the yield potential of wheat has not increased. A large number of respondents agreed that heterosis breeding would be useful for increasing wheat yield. However, little work appears to have been carried out on systematic identification of combiners or of heterotic pools containing divergent germplasm. In wheat, the available male sterility and restorer systems were not considered adequate and seemed to impose yield penalties. Wheat is predominantly a self-pollinated crop and in most of the commercial materials, anthers dehisce before the florets open. For hybrid seed production, adequate level of cross-pollination was considered necessary in this crop. The most critical requirement in production of hybrid wheat seeds was considered to be a stable male sterility/restorer system. However, cost-effective production of hybrid wheat seeds also appeared to require breeding for change in floral structure to develop varieties that allow reasonable cross-pollination. It was stated that such a character would have to be identified amongst cultivated varieties or in the wild relatives of wheat.

Amongst abiotic stresses, heat tolerance, particularly at the terminal stages (seed-filling stage) was considered to be an important breeding goal. Over-expression of *tritacin* gene for improving lysine content was identified as another objective for transgenic research. Some respondents raised a query whether engineering of *b*-carotene pathway in wheat would be useful for addressing the problem of malnutrition. Although *Phalaris minor* is a very recalcitrant weed of wheat crop particularly in the highly productive regions of North-West, only four of the thirteen respondents identified development of transgenics for herbicide resistance as an important objective. The recommendation for herbicide-resistant wheat therefore appeared region-specific. It seems that such transgenics would particularly be useful for the major wheat growing areas of Punjab and Haryana. Many respondents suggested that breeding for specialty traits like bread-making quality and bakery products will allow India to compete in the international markets. These objectives can also be achieved through conventional breeding programmes.

It appears from this survey that the overall maintenance of wheat productivity depends on the incorporation of disease resistance in the existing high-yielding varieties. The breeding community, by and large, seems to feel that enough variability is available to breed for resistance by conventional pure line breeding methodologies. Yield increase can also come through new multi-floret genetic stocks that have been developed at CIMMYT (Mexico) or through heterosis breeding. Heterosis breeding will require male sterility/restorer systems and extensive combining ability studies. In comparison to rice, need for

producing transgenics for meeting major breeding objectives in wheat seems to be less urgent.

Maize (*Zea mays*)

Maize is grown mostly as a rainfed crop in the Indo-Gangetic plains and parts of Southern India. Resistance to stem borer (*Chilo partellus*, *Sesamia inferens*) and the fungal diseases Maydis leaf blight (caused by *Bipolaris maydis* and *Cochliobolus heterostophus*) and Turcicum leaf blight (caused by *Exserohilum turcicum*) were identified as the most important breeding objectives in maize (Figure 1 c). The respondents felt that resistance to stem borer is only available in the wild relatives of *Zea mays* and hence would be difficult to transfer through conventional means. Development of transgenics for stem borer was therefore considered an important goal. Some germplasm resistant to Turcicum leaf blight and Maydis leaf blight were identified by the respondents but it was not clear how much of this germplasm is actually being used for plant breeding.

Maize breeding is done through the development of composites or hybrids. Development of composites is mostly in the public domain. Companies utilizing inbred lines from other countries have developed most of the hybrids that are available in the market. This hybrid maize is not useful for human consumption as it is high in starch content. The respondents felt that there is a need to develop high yielding single cross hybrids that could be used for human consumption. It was stated that male sterility/restorer system is not required in this crop for hybrid seed production as male and female flowers are separate and male florets can be readily removed.

Maize is deficient in two essential amino acids, lysine and tryptophan. Quality protein maize (QPM) high in lysine and tryptophan has already been developed by conventional breeding methodologies. Herbicide tolerance would be useful as maize, being a rainfed crop, has heavy infestation of weeds. Breeders from Punjab specifically recommended the development of herbicide-resistant composites or hybrids. Waterlogging (also referred to as excess soil moisture stress) appeared as the most critical abiotic stress that affects maize cultivation. Due to sensitivity of maize to waterlogging, this crop is not grown extensively in North-Western parts of India as an alternative to rice. As germplasm with adequate resistance to waterlogging is not known, gene discovery for resistance to waterlogging and subsequent development of transgenics were considered high priority areas. However, some specialists were of the view that there are some strains in India which are tolerant to waterlogging. Drought is also a limiting factor in maize cultivation. However, germplasm resistant to abiotic stresses has not been adequately tested under Indian conditions. The need for introducing cold tolerance in winter maize was identified. It was suggested that the germplasm for cold

tolerance could come from the temperate regions of the world. In short, this survey showed that improvement of maize would require both conventional breeding and transgenic approaches. Transgenic approaches would be especially useful for dealing with the problem of stem borer, leaf blight and sensitivity to waterlogging.

Sorghum (*Sorghum vulgare*)

Sorghum (jowar) is both a grain and a forage crop. Sorghum is grown in the rainy season and also in the post-rainy season. This crop is well adapted to grow in rainfed dryland areas. Hybrids in sorghum are extensively cultivated. However, in general, land area under sorghum cultivation is shrinking (Figure 2 c). The post-rainy season sorghum and sorghum grown in 'Kharif' are very important for provision of fodder. The importance of sorghum as a fodder crop for dairy animals is enormous. However, the consumption of jowar grain as bread (or 'roti') has decreased.

Resistance to shootfly (caused by *Antherigona soccata*), grain mould (caused by a number of fungi), stem borer (*Chilo zonellus*), midge (*Contarinia sorghicola*) and charcoal rot (caused by *Macrophomina phaseolina*) were recognized as important problems in cultivation of sorghum (Figure 1 d). Some other problems identified as important in sorghum cultivation included anthracnose (caused by *Colletotrichum graminicola*), smuts (caused by *Sphacelotheca reiliana*, *S. ehrenbergii*, *S. cruenta*, *Tolyposporium ehrenbergii*), downy mildew (caused by *Sclerospora sorghi*) and rusts (caused by *Puccinia purpurea*). Some specialists felt that smuts are not a serious problem and downy mildew and rusts are highly localized problems. It was also suggested that grain mould is a problem only in 'Kharif' when the flowering and grain-set stage coincides with rainfall; shootfly is a problem only in 'Rabi' and if sowing is delayed in 'Kharif'. Some respondents felt that tolerant lines are available to grain mould and shootfly but absolute resistance is not available. The respondents were generally of the view that some germplasm is available for resistance to stem borer, shootfly, charcoal rot resistance, nutritional quality and drought resistance. However, these agronomic traits have not been transferred to good combiners. Low levels of resistance to grain mould and shootfly in early to medium maturing, high-yielding cultivars during 'Kharif' and 'Rabi' seasons were identified as major reasons for poor productivity of sorghum. Hybrids are available only for the rainy season crop in which problems of grain mould and midge are prevalent while the post-rainy season crop suffers from charcoal rot and shoot fly.

It was not clear from the survey whether lack of progress in the utilization of germplasm for breeding-resistant composites or hybrids is due to weak breeding programmes or to complications in transfer due to complex genetics of resistance factors. A number of hybrids are

available for sorghum crop. However, the levels of resistance in male sterile lines to grain mould, shootfly, charcoal rot, stem borer and drought were considered inadequate. A lack of qualitatively superior disease-resistant forage sorghum hybrids was also evident in the response. Requirements of nutritional enhancement in terms of an increase in lysine content, improvement in dough quality and in protein content were identified. The respondents were of the view that incorporation of herbicide resistance into sorghum was not needed. In short, this survey revealed that major objectives for transgenic research in sorghum are to develop lines with resistance to grain mould, stem borer and shootfly.

Pearlmillet (*Pennisetum typhoides*)

Pearlmillet is a crop of dryland areas. However, the area under cultivation of this crop is on the decline as millet grains are losing to rice and wheat (Figure 2 c). The most important yield constraint identified in pearlmillet was susceptibility to downy mildew (*Sclerospora graminicola*) particularly in the single-cross hybrids. Ergot (*Claviceps microcephala*), smut (*Tolyposporium penicillariae*), rust (*Puccinia penniseti*), blast (*Pyricularia setariae*) and mycotoxins in the grain due to fungal infections were identified as major problems in this crop. Amongst abiotic stresses, increased drought tolerance was identified as an important goal.

It appeared that millet hybrids are more susceptible to diseases and the respondents considered incorporation of resistance into divergent combiners by backcross breeding important. To achieve high productivity in pearlmillet, respondents felt that the emphasis on heterosis breeding must continue. The improvement of parental lines of elite hybrids by backcross breeding emerged as an important goal. This survey suggested that genetic diversification of the crop via population improvement should also be emphasized to avoid problems related with narrowing down of the genetic base of the crop. In pearlmillet, hybrids can give 40–50 quintals/ha while the realized yields are in the range of 10–12 quintals/ha. Incorporating the requisite traits, amongst which moisture stress tolerance was considered as an important one, can possibly bridge this gap. It was proposed in this survey that drought-tolerant lines are available in this crop, though there are only a few lines that have been characterized for physiological basis of drought tolerance and the genetics of the trait has also not been worked out. All the respondents suggested development of transgenics for resistance to downy mildew. It was felt that sources that offer a more broad spectrum resistance (horizontal resistance) should be identified and transferred either by sexual crosses or by transgenic approach. The respondents felt that resistance to downy mildew is available and can be incorporated into heterotic combiners. For nutritional enhancement, this survey suggested that vitamin A and protein content could also be improved.

Pigeonpea (*Cajanus cajan*)

Pigeonpea is grown in the rainfed dryland areas of the country in the 'Kharif' season. It was the general feeling of the respondents that the productivity of the crop has not undergone any significant improvement in the past three decades. The most important yield constraint on pigeonpea is from the lepidopteran pest *Helicoverpa armigera*. Another significant insect pest is pod fly (*Melanagromyza obtusa*). Fusarium wilt (caused by *Fusarium udum*), Phytophthora stem blight (caused by *Phytophthora drechsleri* f. p. *cajani*) and sterility mosaic disease were identified as other major constraints on pigeonpea yield (Figure 1 e). Development of extra-early varieties and resistance to drought and waterlogging were identified as important breeding targets. This survey revealed that there were differences in regional priorities: in the Central zone, terminal drought and pod borer (in that order) are important; in the North-East sterility mosaic, wilt, pod fly and pod borer are important and in the North-West, *Phytophthora* blight, susceptibility to cold at the seed filling stage and wilt (in that order) are important. Although a large number of germplasm lines have been identified for resistance to *Fusarium* wilt, sterility mosaic and *Phytophthora* stem blight, resistance for insect pests has been only partial and germplasm with absolute resistance is not available. Some of the respondents suggested wide hybridization for developing resistance to pod borer. *Atylosia scarabaeoides* is resistant to pod borer. However, respondents felt that despite efforts in this direction, no success has been achieved.

Heterosis breeding was identified by sixteen of the eighteen respondents to be the method of choice for increasing the yield of pigeonpea. This survey showed that although combiners are known, no adequate pollination control mechanism was available in this crop. A genetic male sterility (GMS) system developed by ICRISAT (Hyderabad) had been extensively worked upon but had been found inadequate for large scale hybrid seed production as more than 50% of plants were fertile (due to distortion of expected 1 : 1 segregation ratio) and had to be rogued out after identification at the flowering stage. A CMS system has been identified in this crop under the All India Co-ordinated Research Project on pigeonpea. A few fertility restorers have also been found and experimental hybrids have been developed. However, there is an opportunity to use molecular methods for producing male sterile and restorer lines for hybrid seed production in pigeonpea.

A number of physiological traits were identified for improvement in pigeonpea, including improving the harvest index and resistance to drought and waterlogging. Although pigeonpea is essentially a rainy season crop and grows slowly at the seedling stage, only three out of eighteen respondents felt that development of transgenics for herbicide resistance is of any value. In summary, the major emphasis of all the respondents was on develop-

ment of transgenics for resistance to insect pests (all the 18 respondents) and abiotic stress (15 out of 18 respondents). However, the catalogue of abiotic stresses was rather extensive – need for transgenics was felt for resistance to drought, waterlogging, salinity and thermo-insensitivity. While the importance of transgenics for pigeonpea improvement was widely accepted, it is important to consider that there is no reproducible protocol for genetic transformation of pigeonpea. It thus emerged that substantial efforts have to be put in this direction to make use of transgenic technologies for pigeonpea improvement.

Chickpea (*Cicer arietinum*)

Chickpea is a major crop of dryland rainfed agriculture in North India and its cultivation has now spread to peninsular India. The most important priority identified for chickpea breeding was to develop varieties that are resistant to *Helicoverpa armigera* (Figure 1 f). Breeding of chickpea for resistance to wilt (caused by *Fusarium oxysporum* f. p. *ciceri*) and blight (caused by *Ascochyta rabiei*) emerged as the other important goals. The respondents felt that *Fusarium* was a problem both in northern plains and southern regions of the country while *Ascochyta* was mostly prevalent in North.

As chickpea is a self-pollinating crop with a narrow genetic base, there is not much scope for heterosis breeding in this crop. Therefore, pure-line breeding was recognized as a method of choice for developing new chickpea varieties. Due to limited genetic variability available in this crop, the respondents did not feel that there is much hope for overall yield increase. However, stabilization breeding was considered to be of high significance in this crop. Although respondents identified germplasm for blight and wilt resistance, no information could be gained from the survey on the success achieved in the transfer of resistance to elite varieties.

Development of transgenics in chickpea for resistance to *H. armigera* was the major recommendation of all respondents. This survey also showed that despite identification of some germplasm for resistance to wilt and blight, all the respondents suggested development of transgenics for resistance to the two fungal diseases. Development of transgenics for resistance to drought and frost tolerance were also identified as important areas. Response to other goals like nutritional improvement and herbicide resistance was poor. As protocols for chickpea transformation are not optimized fully and the frequency of genetic transformation achieved so far is low, basic work on genetic transformation of chickpea was considered important before breeding objectives outlined above could be dealt with through transgenic technologies.

Mungbean and urdbean (*Vigna radiata* and *V. mungo*)

Vigna radiata and *V. mungo* are the major legume crops

of India. Mungbean yellow mosaic virus (MYMV) was identified as the most critical yield-limiting problem in *Vigna* species. A physiological problem related to sprouting of seeds *in situ* under rains, powdery mildew (caused by *Erysiphe polygoni*) and leaf spot caused by *Cercospora* spp. were the major factors that were identified to limit grain yield (Figure 1 g). It was felt that germplasm for conferring resistance to MYMV, powdery mildew and cercospora leaf spot is available but its utilization has been mainly restricted to develop MYMV-resistant varieties which are now available for all crop growing conditions. The respondents suggested development of transgenics for addressing the problem of MYMV as the highest priority but some respondents differed as they felt that a large number of MYMV-resistant varieties are available and thus transgenics are not needed for this trait. A number of constructs have been developed for pathogen-derived resistance in mungbean and urdbean crop against MYMV. However, low genetic transformation frequencies reported in mungbean and urdbean appear to be a major impediment for developing virus-resistant transgenics. Until reproducible and highly effective transformation protocols are developed for these crops, progress will remain tardy. In general, respondents have overwhelmingly suggested breeding for resistance to insect pests and diseases by transgenic technologies as an important goal. Genetic engineering for resistance to pre-harvest sprouting was another important goal identified in this survey.

Cotton (*Gossypium hirsutum*)

Cotton is the major fibre crop of India. This crop is of great commercial importance to India as it sustains livelihood of a large number of rural people through cultivation and picking and a large workforce employed in both small scale and large industrial units. Cotton apparel is a source of export earnings for the country to the tune of 45,000 crores. The most significant constraints on the productivity of cotton were considered to be insect pests. This study showed that American bollworm (*Helicoverpa armigera*) is the most prevalent and damaging pest of cotton in India. Other lepidopteran pests like pink bollworm (*Pectinophora gossypiella*) and spotted bollworms (*Earias insulana* and *E. vitteulla*) also cause extensive damage (Figure 1 h). Sucking insects such as white fly (*Bemisia tabaci*) and jassids (*Amrasca biguttula biguttula*) too have a major negative impact on cotton yield. Besides insect pests, cotton crop also suffers from bacterial blight (caused by *Xanthomonas malvacearum*) and cotton leaf curl virus (CLCV; a geminivirus spread by whitefly), which has so far affected the crop only in the North-West (Rajasthan, Haryana and Punjab) but may spread to other cotton-growing areas of the country in future. This survey revealed that germplasm is available for early maturity,

bacterial blight resistance and high ginning amongst *G. hirsutum*, extra long staple in *G. barbadense* and for low shedding and early maturity in *G. arboreum* lines. However, resistance to insect pests was reportedly not available in conventional types of cotton.

India was the first country in the world to deploy hybrids in cotton. Hybrid seed of cotton is produced by manual emasculation and hand pollination between good combiners. As no adequate CMS/restorer system is available in cotton, the respondents felt that it would be useful to develop male sterility/restorer system for producing hybrid seed on a large scale. Use of transgenic approaches for hybrid seed production was considered an attainable goal for improving productivity of cotton in the country. Although breeders claimed that a number of combiners for hybrid seed production have been identified, the area under hybrids has declined. Further, it appears that there is a multiplicity of hybrids in the market and only a few of these have survived over the years.

From the point of view of nutrition, a decrease in the content of gossypol in seeds was considered useful for improving the quality of cotton meal. However, it emerged that gossypol will have to be specifically reduced in the seeds as other parts of the plant reportedly require gossypol for resistance to insect pests. All the respondents identified the use of insecticidal proteins obtained from *Bacillus thuringiensis* (*Bt*) for developing cotton transgenics for resistance to lepidopteran insect pests but also cautioned that more than one insecticidal gene would be required as development of resistance to *Bt* toxins has already been shown in some insect species. Resistance to sucking insects through transgenic technologies was identified as another important target. The respondents felt that there is a need to reduce the unsaturated fatty acid content of cottonseed oil and to increase the mono-unsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) contents. Further, increasing oil content above 25% was also considered an important goal. The present varieties have an oil content of around 16–18%. Twelve out of sixteen respondents suggested development of transgenics for herbicide resistance in cotton.

Briefly the most prominent suggestion was the development of transgenic cotton for resistance to insect pests. Oil quality was also an important objective that could be achieved through the development of transgenics. The respondents felt that transgenics for herbicide resistance and male sterility/fertility restoration could help to achieve an increase in cotton productivity. From the survey, it appeared that improvement of cotton in India required a major thrust in the area of development of transgenics.

Potato (*Solanum tuberosum*)

Potato has become an important crop in India both as a vegetable and as raw material for processed food

industry. As potato is vegetatively propagated, heterosis can be fixed readily by propagating F₁s. No male sterility systems are required for potato. At the national level, resistance to late blight disease (caused by *Phytophthora infestans*) was identified as the most important breeding objective followed by mosaic disease (caused by a large number of viruses), bacterial wilt (caused by *Ralstonia solanacearum*), black scurf (caused by *Rhizoctonia solani*) and aphids (Figure 1 i). Aphids are more of a problem as vectors for viral transmission. In terms of severity of diseases and pests, there appeared major regional variations. In the main potato growing areas of Indo-Gangetic plains, late blight and black scurf were considered as the major problems. In the North-Western hills, late blight appeared as an important disease and in the Eastern hills both late blight and wart disease affected potato crop. In the plains of Maharashtra and Karnataka, mosaic virus and foliar necrosis were considered as the major problems. Late blight appeared to be a major problem in the Southern hills and nematodes extensively damaged the crop in the Nilgiri hills.

The response of specialists showed that many of the wild relatives of *Solanum* carry genes for resistance to the major pests and pathogens. However, as no query on the effective utilization of germplasm for improvement of crops was included in the survey, it is difficult to say how effective the wide hybridization programmes are in this respect.

Thirteen out of nineteen respondents suggested breeding for nutritional enhancement of potato. Suggestions included expression in tubers of provitamin A and increasing the content of sulphur containing amino acids. The *ama1* gene (that encodes a seed storage protein having a balanced amino acid composition) from *Amaranthus* has been mobilized into a large number of potato varieties in India. Eighteen out of nineteen respondents suggested development of transgenics to control biotic stresses mostly fungal diseases in potato. Development of transgenics resistant to tuber moth appeared to be an important suggestion since no germplasm is available for resistance to tuber moth. Twelve of the nineteen respondents suggested development of transgenics for abiotic stresses as an important goal. Traits to be dealt with included heat tolerance, particularly to develop cultivars, which can form tubers above 20°C. However, development of transgenics for tuber formation under high temperature would require a thorough understanding of induction and development of the process at the molecular level. For value addition, it was proposed by some respondents that varieties with more than 20% dry matter and less reducing sugars should be developed.

It was proposed in this survey that yield improvement in potato could be attained by utilizing diverse genetic base available in *Solanum tuberosum* ssp. *andigena* (for biotic and abiotic stresses) through introgression breeding programmes. Crossing unrelated genetic material by a two-way hybridization approach followed by vegetative

propagation was considered adequate for enhancing yield. In general, potato researchers seemed to favour use of transgenic technologies for meeting a number of breeding objectives that included development of cultivars resistant to fungal and viral diseases and high temperature. It appears that potato transgenics are specially needed for increased starch production through over expression of suitable genes, reduction of cold storage induced sweetening, production of vaccines for livestock, development of aphid-(vector) resistant cultivars, development of late blight-resistant cultivars, gene pyramiding for durable resistance to insect pests and development of temperature insensitive genotypes.

Indian mustard (*Brassica juncea*)

Indian mustard (*Brassica juncea*) is a major oilseed crop of rabi season in Northern India and is largely grown as a rainfed crop. It is predominantly a self-pollinated crop but considerable cross-pollination also occurs. There is a real possibility of development of hybrids in this crop but this potential has remained untapped. No commercial hybrids have yet been released in this crop but some field trials have been conducted on experimental hybrids based on the use of transgenic male sterile and restorer lines.

The most important breeding objective in mustard is the development of lines with resistance to *Alternaria* blight (caused predominantly by *Alternaria brassicae* and *A. brassicicola*), aphids (*Lipaphis erysimi*) and white rust (*Albugo candida*) (Figure 1 j). The respondents agreed in general that resistance to white rust is available within *B. juncea* germplasm but sources of resistance to *Alternaria* are not available. This survey revealed that development of transgenics for resistance to *Alternaria* would be a major breakthrough for mustard cultivation. It would be useful to consider if disease resistance conferring genes of *Arabidopsis thaliana* and/or of other alien species could be used in mustard for resistance to *Alternaria* blight. Aphids cause huge yield losses in mustard crop. It emerged that effective strategies were not available to control this pest, except to breed for early maturing cultivars at the cost of yield. More or less, all the respondents suggested exploration of transgenic technologies for developing aphid-resistant cultivars.

Improvement in oil quality by developing 'zero' erucic acid and meal quality by developing 'zero' glucosinolate cultivars (less than 30 µ moles of glucosinolate per gram of defatted meal) were identified as major breeding goals. Resistance to herbicides was not considered important in this crop. Some respondents proposed that mustard transgenics could be developed for resistance to abiotic stresses like drought, heat, cold and salinity. *Brassica* transgenics have already been developed in the country with *codA* gene and would need to be tested for resistance to different abiotic stresses at field level. Heat

tolerance for early planting and at seed filling stage could also be of value and the possible role of heat shock proteins (HSPs) in this context could be examined. It was proposed that heterosis breeding could substantially increase mustard yield. A complete male sterility/restorer based on transgenic technologies has been developed both in private and public systems and could be used for developing hybrid seeds. Mustard and other *Brassica* species are highly amenable to techniques of genetic engineering. Thus, there is tremendous scope for improvement in the mustard crop by the use of transgenic technologies.

Soybean (*Glycine max*)

Soybean is an important legume crop of USA, Brazil, China and Argentina. In India, this crop is currently grown in large tracts of Madhya Pradesh and other parts of Central India during the 'Kharif' season as a rainfed crop. Soybean is also a very popular crop in the Northeastern states, particularly Nagaland, from very early times. It is an introduced crop and initially, was free of diseases. However, continuous cultivation of this crop has led to incidence of a number of diseases on the crop. In the survey, the most important problem in soybean cultivation appeared to be yellow mosaic virus (YMV) (Figure 1 k), a geminivirus, spread through whiteflies. Besides YMV, soybean rust (caused by *Phakopsora pachyrhizi*) was identified as an important disease and diseases such as root rot (caused by *Macrophomina phaseolina*), pod blight (caused by *Colletotrichum* sp.) and bacterial blight (caused by *Xanthomonas campestris* pr. *glycines*) were considered noteworthy. YMV is the predominant disease in Northern plains and hills but is not a threat in the major soybean growing areas of Madhya Pradesh. Availability of resistant lines would allow cultivation of soybean in place of rice in the Northern plains.

The most important recommendation in soybean was to develop transgenics that are resistant to YMV and fungal diseases. Amongst insect pests, stem fly (*Melanogromyza sojae*), stem borer (*Oberlopsiis brevis*) and green semi-looper (*Chrysodeixis acuta*) were identified as major problems but exact quantification of yield losses due to these insect pests remains to be done. The respondents in general felt that lack of certified seed and poor agronomic practices are contributing in a significant way towards low yield of soybean. As a 'Kharif' crop, soybean has major problems with weed infestation. However, only three respondents proposed development of transgenics for herbicide resistance in this crop. The respondents also felt that protocols for genetic transformation of soybean need to be established in the country if transgenics for the identified breeding objectives are to be developed.

Epilogue

Two major crops namely groundnut and sugarcane could not be covered in this survey. These two crops along with some other oilseed crops like safflower, sunflower and sesame will need to be included in future assessments. It would also be useful if annual losses incurred due to lack of adequate resistance to biotic and abiotic stresses are estimated for different crops.

Finally, all the conclusions and suggestions made in this article are based on analyses of inputs that were received from different respondents and are not the personal views of the authors. It is possible that some of the points made in this write-up represent the opinions of individual contributors. We have not carried out any statistical validation of the received inputs. We only wish to add that the suggestions made in this article represent the collective wisdom of a large number of ground-workers and therefore, must be further investigated by those who direct the future of Indian biotechnology/agriculture research.

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