Journal of Applied Sciences

ISSN 1812-5654

Journal of Applied Sciences 10 (17): 1885-1893, 2010
ISSN 1812-5654

Novel Approaches and Techniques of Cost-saving Potential in Crop Improvement

S.K. Sinha, S. Das, R.C. Misra and B.R. Singh
Department of Plant Breeding and Genetics, College of Agriculture,
Orissa University of Agriculture and Technology, Bhubaneswar-751003, Orissa, India

INTRODUCTION

Crop improvement is cost-intensive. It demands in ample measure inputs like money and manpower, land and labour and most important of all, time. Though apparently a non-monetary input, time contributes to cost in diverse ways. Breeding improved crop varieties through conventional approaches and techniques is slow. New approaches and novel techniques need be adopted to counteract constraints of cost and time (particularly, in resource-poor situations), which will also help to enhance efficiency in crop improvement. Rapid progress in cereal improvement in recent times through the ideotype approach and the use of *in vitro* culture of plant tissue for accelerating crop improvement in general illustrates this point. Even simple or small innovations can appreciably contribute to saving time and cost in breeding crops, particularly those of long duration. A few potentially useful approaches and techniques are briefly discussed below, basing on our work and experience in different crops. Hopefully these will contribute a cost-effectiveness in crop improvement.

THE USE OF MINIATURE PLANTS

Roots can be induced from isolated leaves of many plants using auxins. When planted in pots these rooted leaves can be maintained for a long time as miniature plants, which show certain aspects of growth and development like secondary growth, lamina expansion (as in *Basella*), bud formation (as in *Vitis*), tuberization (as in Sweet potato), nodulation (as in legumes) etc. (Samantarai and Sinha, 1955). These phenomena differ depending on species. Intra-species variation has also been observed.

As suggested long back by Sinha (1964), the miniature plants can be put to multiple uses in potato breeding. A simple lab technique was developed for screening potato varieties for nematode resistance using rooted leaflets. Thus a lot of labour, time and field space could be saved (Nirula and Pushkarnath, 1966). This simple technique is also useful in breeding for disease resistance (Poehlman and Borthakur, 1969). Through the use of miniature plants, it should be possible to undertake laboratory evaluation of germplasm for a number of characters (e.g., resistance to different pathogens and pests and physiological characters like abiotic stress tolerance, photosynthetic efficiency, mineral stress response etc.) along with simultaneous field evaluation for agronomic characters of breeding interest.

The miniature plant approach can be profitably used for other tuber crops, legumes (for evaluation of germplasm for nodulation and nitrogen-fixation capacity) and tree species. There is one limitation however, since all plants are not amenable to induction of rooting by auxins. To this recalcitrant group belong most monocots and a few dicots.

THE USE OF SHORT-TERM LEAF CULTURE AND DARK RESPONSE

The limitation due to lack of rooting response of most monocot leaves to auxins can be at least partially circumvented through the use of short-term culture. Infact, excised cereal leaves can be easily cultured for a very short period of at least 72 h using simple culture media (contrasted with complex media for the *in vitro* tissue culture). In this context, another well-known phenomenon thought to be useful is dark-induced bleaching or etiolation. Light regulates the expression of a number of chloroplast genes and also certain nuclear genes associated with chloroplast development and function. Hence, absence of light affects chloroplast development and causes etiolation. Grover *et al.* (1986) examined changes in various components of photosynthetic activity during the dark-induced senescence of detached wheat leaves. Saulescu and Kronstad (1998) found large and visible genotypic differences in dark-induced senescence in wheat by exposing seedlings of wheat cultivars at the 2-leaf stage to continuous darkness. They also observed that chlorophyll content decreased linearly with dark treatment

Corresponding Author: S. Das, Department of Plant Breeding and Genetics, College of Agriculture,
Orissa University of Agriculture and Technology, Bhubaneswar- 751003, Orissa, India

duration and the regression slopes varied significantly among varieties. Saulescu *et al*. (1998) studied genotypic differences in dark-induced senescence among fourteen hybrids of maize and reported that dark-induced senescence symptoms were correlated with field stay green scores (the percentage of green plants at physiological maturity) of maize hybrids.

The dark response approach can also be adopted for GP (germplasm) evaluation for breeding cereals, using intact seedlings. Intra-species variation in etiolating, whether *in vitro* or *in vivo* is an unexplored area. Saulescu *et al*. (2001) conducted a study to investigate the feasibility of a seedling test for estimating genotypic differences in senescence rate of wheat. They studied the response of several wheat genotypes to dark exposure of seedlings, by measuring the rate of chlorophyll loss in the first leaf. They found that the rate of chlorophyll loss varied significantly among genotypes, the highest rate being more than double of the lowest. Linear regression accounted for most of the chlorophyll content variation during dark treatment in all cultivars. They concluded that a seedling test for dark-induced senescence is a potential tool in breeding wheat for optimum senescence pattern. For physiological characterization of stay green mutants in durum wheat, Spano *et al*. (2003) excised the fifth youngest fully expanded leaves after booting and incubated the excised leaves in dark at 20°C for 6 days. They excised the leaves of both parental and mutant plants. Leaves were sampled every 3 days for chlorophyll content. They found that the chlorophyll content of mutant plants were more than the parental plants (cv. Trinakaria), indicating that senescence of detached youngest leaves in darkness was faster in parental line than in the mutants. The chlorophyll contents, measured by using SPAD meter, of mature flag leaves of control plants grown in the green house also decreased much earlier in parental line than in mutants. The yield data indicated that mutant lines had higher seed weights and grain yield per plant than the parental line. They concluded that dark response might indirectly help in selection of high yielding genotypes. Saulescu and Mustatea (2002) reported that a good correlation existed between the rate of dark-induced chlorophyll loss in wheat seedlings and the rate of age-induced senescence of flag leaves in the field in absence of disease. Slow senescence seems to be a characteristic of wheat cultivars adapted to more favourable environments, whereas fast senescence can be found in cultivars that are more adapted to stress environments. The seedling test for dark-induced senescence could facilitate selection for optimum senescence pattern suitable for target environment.

Fig. 1: Genotypic differences in dark-induced senescence in rice (*Oryza sativa*)

The relevance of dark induced bleaching in breeding has been indicated in our laboratory in rice and ragi (Fig. 1). Pattanaik (1994) examined dark-response of nineteen short duration ragi varieties and its bearing on adaptation. To study dark-response, fully expanded first leaves were excised from 7-day old seedlings and incubated in dark for 48 h. Senesced leaves were scored as: green (score 0), partially green or yellow (score 1) and fully yellow (score 2). Dark response of a variety was evaluated by an etiolation index, derived by dividing the total score by twice the number of leaves. On the basis of etiolation index (EI) value, the nineteen varieties were classified into 3 dark-response classes - low, moderate and high. MLT yield data were used to evaluate the adaptation pattern of varieties. It was observed that varieties falling in moderate dark-response class showed most desirable pattern of adaptation. He concluded that genotypic differences for dark-induced senescence could be used to identify high-yielding short duration ragi varieties with desirable type of adaptation.

Mohapatra (1997) studied dark-response of 14 medium and 9 late duration ragi varieties in both *in vivo* and *in vitro* conditions. In *in-vitro* condition, fully expanded first leaves were excised from 7 days old seedlings and exposed to dark for a period of 48 h. In *in vivo* condition, 7 day old seedlings were incubated in dark for 48 h and senescence of fully opened first leaves were recorded. Dark-response of varieties were determined by calculating etiolation index in both *in vivo* and *in vitro* conditions. On the basis of *in vivo* EI and *in vitro* EI values, the varieties were classified as Dark-S (Sensitive), Dark-MR (moderate response) and Dark-R (resistant). In the medium duration group, the Dark-MR and Dark-R classes contained all top yielders and most above-average yielders (yield data from All India Coordinated Trial was used for field evaluation). In the late duration group, Dark-S class contained more above-average yielders and top yielders than Dark-R or Dark-MR class. He suggested that dark response (Dark-induced senescence) could be used to identify high- yielding varieties.

Das *et al*. (2008a) studied dark response of thirty six rice genotypes and found that high sensitivity to dark treatment in terms of senescence (SI) index may indicate better adaptation to rich environments and low sensitivity to dark treatment may indicate better adaptation to poorer environments.

PREFERENTIAL INDUCTION OF MICRO-MUTATIONS (PIMM)

Gregory (1956) focused attention on the utility of induced polygenic variation or micro-mutations involving quantitative characters for improvement of yield. The conventional method adopted for this purpose comprises elimination of macro-mutatants and selection of only normal looking plants and evaluation of lines derived from them for yield and desirable agronomic traits. Micro-mutation breeding thus entails large investment of many inputs and carries a huge load of garbage to be shed gradually through field testing over generations. Hence arises the need of preferential induction of mciro-mutations, i.e., without accompanying macro-mutations.

In an attempt to economize and accelerate breeding of late duration winter rices, Maleic Hydrazide (MH) was found useful as a mutagen through PIMM, which helps to eliminate wastage in mutation breeding for yield. This novel technique was developed by Mahapatra (1967) and used for rapid improvement of a number of popular rice varieties. In each case, an appreciable number of higher yielding micro-mutants (HYM) could be produced in a much shorter period and with much less cost than otherwise possible by conventional method.

Most gratifying results were obtained with the popular winter rice, T90. Among several HYMs, three out yielded the parent by more than 20% and one gave 29% higher yield. This suggested the potential of PIMM, which may not be realized in all cases, since the response to MH varies with the genotype (due to genetic control of mutability). The degree of response to the mutagen MH can be ascertained through preliminary seedling growth response. In fact, in one study T90 was most sensitive (Das and Sinha, 1965). Das and Sinha (1965) treated seeds of nineteen varieties of rice with 25 and 100 ppm aqueous solution of MH for 24 h and also seeds were soaked in water to serve as control. Growth response of seedlings was studied at regular intervals for five days. They found that in most varieties growth of shoots and roots at any interval was retarded due to treatment with MH and the maximum was recorded at 100 ppm after 5 days. Variation was found in varietal response to MH. In case of variety T 90, root and shoot growth with 100 ppm MH after 5 days was 40 and 70%, respectively, of the control. The variety T 1242 appeared to be quite tolerant to MH, in which 5 days root growth with 25 and 100 ppm MH were 108 and 96%, respectively, of the control and the corresponding shoot growth were 109 and 125%. The differential response of varieties pointed to a genotypic control of sensitivity to MH. They also found that MH-resistant varieties were high yielder than sensitive varieties.

Dash (1988) studied the response of wheat varieties to MH (250, 500 and 1000 ppm) in the laboratory. Observations were recorded on shoot length and length of the longest root. Using numerical taxonomic method, the varieties were classified as moderate MH-response and extreme- response groups. To compare the results of laboratory study, a field trial was conducted. It was observed that moderate-response types were moderately stress- tolerant (heat/drought) in the field and extreme-response types were extreme in stress response (being more tolerant or more sensitive). Das *et al*. (2008b) tested MH sensitivity of 36 rice and 30 ragi genotypes in terms of growth inhibition index to predict yield and adaptability at early seedling stage. Laboratory result was compared with multilocation trial result and it was suggested that low sensitivity to MH could be used as an indicator of high yield potential.

PIMM can also be applied to high yielding varieties of rice and other self-pollinated cereals like wheat as suggested by Sinha and Mohapatra (1994). Its potential use for other crops is yet to be tested. Preferential attack of MH on heterochromatin makes it possible for a cytological prediction of the quantum of improvement through PIMM. The tomato genome with high heterochromatin content might be most responsive to PIMM. The importance of cytology in this context is emphasized.

NOVEL USE OF CHEMICALS

The antibiotic streptomycin: It is known since long that streptomycin (SM) induces bleaching/albinism in barley (Von Euler, 1947). It brings about ultra structural changes at the cellular level (Behn and Arnold, 1974) and causes induction of cytoplasmic organellar mutation including male-sterility (Burton and Hanna, 1982). It also affects gene expression by virtue of its effect on the translation process during protein synthesis. The diverse effects of this chemical has been thoroughly reviewed by Kirk and Tilney Bassett (1978).

SM can induce mutations in plants. Maize plants treated with 0.1 to 0.2% SM for 48 h produced multi-tillering mutant lines in maize (Choudhary and Minocha, 1989). SM treatment in sorghum produced mutants with improved capacity for phosphorous utilization (Spivakov and Klimashevski, 1989). Plants exposed to SM showed reduction in growth (Kirk and Juniper, 1955; Gerhard, 1967; Mukherjee and Bag, 1975; Nageswar Rao *et al*., 1980) and chlorophyll synthesis (Khudairi, 1961; Babayan *et al*., 1975; Mancinelli *et al*., 1975: Pretova, 1980; Zubko and Dey, 1998). SM is mostly used in somatic hybridization as a chloroplast marker for selection of fusion products (Kinoshita and Reiko, 2001).

However, its possible use in crop improvement was realized much later when evidence for genetic variation in SM-induced bleaching was observed in maize in our laboratory (Sinha and Satpathi, 1977). Inbred lines of maize showed variation in SM-response, which could be roughly measured, using a Bleaching Index (BI) developed by us. Thus genotypes of maize and other crops could be characterized for SM-sensitivity.

Of what use however is such characterization? The finding in maize gained further significance, when rice varieties were characterized for bleaching response (Sinha and Satpathi, 1977). It was of great interest to note that semi-dwarf High Yielding Varieties (HYV) were highly SM-sensitive and tall low yielding varieties (LYV) were resistant. This important finding was confirmed, using a large number of varieties in repeated experiments (Satpathi, 1991). It was inferred that screening germplasm of rice for SM-response may have some practical value.

There are two categories of characters for which germplasm characterization and evaluation is rather deficient: (1) physiological characters and (2) cytoplasm characters/organelle behaviour. A simple and rapid lab technique like bleaching response to SM would hence be of some value. Satpathi (1991) made a comparison of bleaching response of several crops (major cereals, millets, pulses and oilseeds), using a few varieties for each crop. Certain findings of these studies are worth mentioning (1) C_4 species are generally more SM-sensitive than C_3 species (2) There are however, exceptions, particularly in C_3 crops, which is of some significance (for example. SM-sensitive rice varieties may have high yield potential), (3) Drought tolerant species and varieties are highly SM-resistant Horsegram which is highly drought tolerant is almost SM-resistant.

The earlier finding as to HYV-LYV difference in bleaching response to SM (Sinha and Satpathi, 1977) formed the basis of a laboratory approach to an indirect (laboratory) evaluation of adaptation, in subsequent studies on different crops e.g., rice, wheat, ragi and green gram. Rath (1977) examined the possible relationship between SM-induced albinism in seedlings and high yield in wheat varieties and found that high yielding varieties of wheat were sensitive to SM.

Sinha and Swain (1978) used SM-induced bleaching as a criterion of selection in seedling stage of EMS-derived populations of ragi (Fig. 2). They found that the SM-sensitive lines were earlier in maturity, shorter in height and higher yielding than SM-resistant lines. Das and Sinha (1986) analysed SM-sensitivity of 22 mutant lines of a finger millet strain (T_{25-1}) at an early seedling stage. To compare the results of laboratory study, a field trial was conducted. Correlation analysis between SM-response and plant characters indicated that SM-sensitivity in terms of bleaching index was positively correlated with maturity duration and plant height. They suggested that selection for SM-sensitivity in seedling stage might lead to selection of lines with shorter height, earlier maturity and higher number of tillers per plant. Selection for SM-resistance may lead to selection of late maturing lines with taller height and fewer tillers.

SM-response could be used as a criterion in preliminary laboratory evaluation of broad adaptation pattern of new high-yielding rice varieties and for germplasm screening for drought tolerance (Das and Sinha, 1992). The high-yielding varieties of wheat having low sensitivity to SM in terms of seedling growth were found to be of general adaptation (Das and Sinha, 1995). Sinha *et al*. (1996) while working in ragi suggested that it was possible to identify ragi varieties having general adaptability by selecting the low SM-response class. In selecting greengram genotypes for bleaching sensitivity and resistance to growth inhibition, the selected group is likely to contain high frequency of superior genotypes with desirable pattern of adaptation (Singh and Nanda, 1997). Das (2001) found that the high yielding varieties of rice having low sensitivity to SM in terms of bleaching response were drought tolerant. Das (2008) evaluated SM sensitivity of 36 rice and 30 ragi genotypes in terms of

Fig. 2: Genotypic differences in SM-induced bleaching effect of ragi (*Eleusine coracana*)

Fig. 3: Genotypic differences in SM-induced bleaching effect of rice (*Oryza sativa*)

bleaching index (Fig. 2, 3). To compare the results of laboratory study, multilocational trials were conducted. And it was suggested that high sensitivity to SM could be used as an indicator of high yield potential.

Use of gibberellic acid (growth regulators): The use of plant growth regulators as an aid to screen crop germplasm has been reported by many authors. Sip *et al*. (1986) used Gibberellic Acid (GA) to identify desirable genetic dwarfs in wheat. Obrein and Pugsley (1981); Boyd and Wade (1989) suggested the use of GA to screen wheat germplasm for yield potential. The relationship between GA response and rust resistance in wheat reported by Syme and Thompson (1986) would suggest the possible utility for screening for GA response in indirect evaluation of certain types of disease resistance. As an aid to rapid evaluation for dwarfism, seedling tests involving GA have been developed in rice (Matsuanger *et al*., 1981).

Use of digitonin: Singh *et al*. (2008) studied the digitonin sensitivity of high yielding mungbean varieties. Sensitivity to this chemical was measured by using a suitable index based on visual rating of abnormal hypocotyls growth. They found that high sensitivity to digitonin could be used as an indicator of high yielding ability and adaptation to favourable environments.

Of the different chemicals suggested here for chemo-response studies one or more could be used. Being well tested in different crops, SM is a chemical of choice, which may be supplemented with one or more of the remaining for better productivity. These studies showed that the simple lab technique (seedling response to chemicals) helps in preliminary selection of (1) varieties with high yield potential and (2) stable - yielding varieties with a greater probability. No other techniques is available today to be used as a simple, low-cost alternative to the cost-intensive field evaluation through multilocation/ environment testing.

NOVEL USES OF NUMERICAL TAXONOMY (NT)

Orthodox taxonomy or classification as is usually practiced basing on a few qualitative characters is of little application in crop improvement except for correct identification of plants. Numerical Taxonomy (Taxometry), which adopts a mathematical approach to classification, can make use of any number of quantitative characters besides qualitative characters. Its applicability in crop improvement, which deals with both kinds of characters, is evident. One use that has been suggested (Sneath and Sokal, 1973) and often adopted is selection of parents for hybridization in breeding for higher yield.

We have conceived and shown much wider application of NT in problems of crop improvement e.g., evaluation of adaptation (Das and Sinha, 1992, 1995), mutation breeding (classification of micro-mutants and mutagens; Singh and Sinha, 2010; Muduli and Misra, 2008), classification of environments (trial sites/environment in multilocation testing, crop zonation etc., Karamura, 1998), maintenance of genetic purity, cytogenetic studies related to breeding (Badr and Elkington, 1978; Szlachciak and Boron, 2003; Jackson and Crovello, 1971), evaluation of inbreds for heterosis breeding (Qi *et al*., 2010; Koutsos *et al*., 2000; Fan *et al*., 2009) use in resistance breeding (Kim *et al*., 2004; Karuri *et al*., 2009), variety identification (Portis *et al*., 2004; Saric-Kundalic *et al*., 2009; Shuaib *et al*., 2007) and other possible uses (in germplasm collection and utilization, growth pattern analysis to detect sub-types within plant types etc).

NT may be especially useful in management of massive data as in the case of All India Coordinated Trial (AICT) for different crops. We strongly feel more information, relevant to breeding could be extracted from these data sets than is done at present. NT can thus help in effective utilization of data, assiduously gathered with heavy investment. The significance of the AICT data stems from one important aspect of NT, which is the principle that "the greater the information content in the taxa of a classification and the more characters on which it is based, the better the classification will be" (Sneath and Sokal, 1973). The term "better" means greater productivity, which should be a goal in plant breeding research. The goal, however, is difficult to achieve, in any case, AICTs do provide a large number of characters to work towards a predictive (more useful) classification of the taxa (varieties/locations or sites of testing etc.).

MOLECULAR MARKERS

The role of molecular markers is a novel and recent approach in crop improvement. Among the various markers, RAPD (Random amplified polymorphic DNA) is methodologically simple and less expensive. Therefore, possible use of RAPD markers was tried in our lab for preliminary evaluation of yield and adaptability. Das *et al*. (2008c) studied the relationship of RAPD banding pattern with yield potential and adaptability of rice genotypes. The experimental results revealed that absence of polymorphic band ~400 bp in OPD-08 gave indication about high yield potential; presence of polymorphic band ~750 bp in OPD-05, ~400 bp in OPD-08 and ~2000 bp in OPD-18 gave indication about genotypic adaptation to poorer environments and absence of these markers gave indication about genotypic adaptation to rich environments. The experimental investigation of Das *et al*. (2009) revealed that the presence of OPA4~1800 bp and absence of OPN15~900 bp bands in finger millet may give some indication about specific adaptability of genotypes to poor environments. Absence of OPA4~1800 bp and presence of OPN15~900 bp in finger millet would indicate specific genotypic adaptation to rich environments. Finally they concluded that these studies may help to predict the genotypic adaptation of different genotypes before going for multilocation trial.

Epilogue: It is not possible to present all potentially useful approaches/techniques, except for a few important observations. We conclude with our conviction that integration of the novel and the conventional approaches/techniques can contribute to cost-effectiveness in crop improvement. This economic consideration should also be the concern of plant breeders and germplasm workers.

REFERENCES

Babayan, R.S., A.M. Gevorkyan and N.A. Soakyan, 1975. Effect of streptomycin on chlorophyll biosynthesis in wheat seedlings. Fiziol. Rast., 22: 484-489.

Badr, A. and T.T. Elkington, 1978. Numerical taxonomy of species in *Allium* subgenus *Molium*. New Phytol., 81: 401-417.

Behn, W. and C.G. Arnold, 1974. The effect of streptomycin and neamine on the structure of chloroplasts and mitochondria in *Chlamydomonas reinhardii*. Protoplasma, 82: 77-89.

Boyd, W.J.R. and L.J. Wade, 1989. GA insensitivity as an aid to selection for high yield in wheat. Proceedings of the Australian Conference on Plant Breeding, (ACPB'89), Adelaide, South Australia, pp: 323-324.

Burton, G.W. and W.W. Hanna, 1982. Stable cytoplasmic male-sterile mutants induced in cultivar Tift 23DB1 pearlmillet with mytomycin and streptomycin. Crop Sci., 22: 651-652.

Choudhary, V.K. and G.L. Minocha, 1989. Studies of some induced morphological mutants. Maize Genet. Cooperation News Lett., 63: 91-98.

Das, P.D. and S.K. Sinha, 1965. Variation in seedling response of rice varieties to maleic hydrazide. Genetics, 52: 439-445.

Das, P.K. and S.K. Sinha, 1986. Relation of a seedling character to yield and yield components in finger millet (*Eleusine coracana*). J. Orissa Bot. Soc., 8: 49-50.

Das, S. and S.K. Sinha, 1992. Use of streptomycin as a chemical aid in the laboratory evaluation of broad adaptation patterns in rice. Pl. Ski. Res., 14: 17-20.

Das, S. and S.K. Sinha, 1995. Physiotaxometric studies of adaptation in wheat: I A. Classification of genotypes based on seedling growth response to streptomycin. Curr. Agric. Res., 8: 101-106.

Das, S., 2001. Laboratory screening of high yielding rice varieties for drought tolerance. Environ. Ecol., 19: 14-18.

Das, S., 2008. Comparative study of field and laboratory evaluation of adaptation in rice and ragi varieties. Ph.D.Thesis, Orissa University of Agriculture and Technology.

Das, S., R.C. Mishra and S.K. Sinha, 2008a. Variation in dark induced senescence pattern of rice genotypes. Proceedings of the National Seminar on Climate Change and Natural Resource Management, Mar. 17-18, OUAT, Bhubaneswar.

Das, S., R.C. Mishra, G.R. Rout and S.K. Sinha, 2008b. Relationship of RADP banding pattern with yield potential and adaptability of mid-early rice genotypes. Int. J. Integrative Biol., 2: 109-115.

Das, S., S.K. Sinha and R.C. Mishra, 2008c. Variation in seedling growth inhibition due to maleic hydrazide treatment of rice (*Oryza sativa*) and ragi (*Eleusine coracana*) genotypes and its relationship with yield and adaptability. J. Crop Sci. Biotech., 11: 215-222.

Das, S., R.C. Misra, G.R. Rout, M.C. Pattnaik and S. Aparajita, 2009. Relationship of status of RAPD polymorphic bands with genotypic adaptation in early finger millet genotypes. Afr. Crop Sci. J., 17: 61-69.

Dash, P.K., 1988. A numerical taxonomic study of seedling physiotypes in breeding wheat for high temperature tolerance. M.Sc. Thesis, Orissa University of Agriculture and Technology.

Fan, X.M., Y.M. Zhang, W.H. Yan, H.M. Chen and J. Tan *et al*., 2009. Classifying maize inbred lines into heterotic groups using a factorial mating design. Agron. J., 101: 106-112.

Gerhard, H., 1967. Effect of antibiotics on growth and ion uptake of *Zea mays*. Ber. Schweiz. Bot. Ges., 77: 210-256.

Gregory, W.C., 1956. Induction of useful mutations in the pea nut. Brookhaven Symp. Biol., 9: 177-190.

Grover, A., S.C. Sabat and P. Mohanty, 1986. Effect of temperature on photosynthetic activities of senescing detached wheat leaves. Plant Cell Physiol., 27: 117-126.

Jackson, R.C. and T.J. Crovello, 1971. A comparison of numerical and biosystematic studies in *Haplopappus*. Brittonia, 23: 54-70.

Karamura, D.A., 1998. Numerical taxonomic studies of the East African high land bananas (Musa AAA-East Africa) in Uganda. Ph.D. Thesis, Department of Agricultural Botany, University of Reading.

Karuri, H.W., E.M. Aleka, R. Amata, A.B. Nyende and A.W.T. Muigai, 2009. Characterization of Kenyan sweet potato genotypes for SPVD resistanceand high dry matter content using simple sequence repeat markers. Afr. J. Biotechnol., 8: 2169-2175.

Khudairi, A.J., 1961. Effect of streptomycin on *Xanthium*. Biochem. Biophys. Acta, 46: 344-354.

Kim, M.S., S.J. Brunsfeld, G.J. Mc Donald and N.B. Klopfenstcia, 2004. Effect of white pine blister rust and rust resistance breeding on genetic variation in Western pine (*Pinus monticola*). TAG, 106: 1004-1010.

Kinoshita, T. and T.S. Reiko, 2001. Streptomycin resistance of rice varieties and dwarf lines. Rice Genet. Newslett., 5: 13-14.

Kirk, J.T.O. and B.E. Juniper, 1955. The Plastids, their Chemistry, Structure and Inheritance. 1st Edn., Elsevier Science Ltd., North Holland.

Kirk, J.T.O. and R.A.E. Tilney-Bassett, 1978. The Plastids, their Chemistry, Structure, Growth and Inheritance. 2nd Edn., Elsevier Science Ltd., North Holland, ISBN-10: 0444800220, pp: 980.

Koutsos, T.V., M. Koutsika-Sotiriou, E. Gouli-Vavdinoudi and K. Tertivanidis, 2000. Study of the genetic relationship of Greek okra cultivars (*Abelomoschus esculentus* L.) by using agronomic traits, heterosis and combining ability. J. Veg. Crop Prod., 6: 25-35.

Mahapatra, R.K., 1967. Effects of chemical mutagens on rice. Studies on mutagenic action of maleic hydrazide and caffeine. M.Sc. Thesis, OUAT, Bhubaneswar, Orissa.

Mancinelli, A.L., C.H. Yang, P. Lindquist, O.R. Anderson and I. Rabino, 1975. Photocontrol of anthocyanin synthesis III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant Physiol., 55: 251-257.

Matsuanger, K., K. Manuyama and H. Iketashi, 1981. Semi-dwarfism and varietal response to exogenous gibberellic acid in rice. Oryza, 18: 208-213.

Mohapatra, B., 1997. Variation on plastid behaviour and chemical induction of plastid mutation on ragi. M.Sc. Thesis, Orissa University of Agriculture and Technology.

Muduli, K.C. and R.C. Misra, 2008. Genetic divergence analysis among micromutant lines in finger millet (*Eleusine coracana* G.). J. Crop Sci. Biotechnol., 11: 63-68.

Mukherjee, S. and A. Bag, 1975. Metabolic alternation in rice seeds germinating in streptomycin sulphate. Bull. Bot. Soc. Bengal, 29: 113-120.

Nageswar Rao, A.V., V. Hanumantha Rao and K. Rameswar Rao, 1980. Phyto toxicity of antibiotic and its reversal by gibberellic acid in germinating rice seeds (*Oryza sativa*). Ind. J. Bot., 3: 65-69.

Nirula, K.K. and Pushkarnath, 1966. A technique for screening tuber-bearing *Solanums* for resistance to root knot nematodes. Euphytica, 15: 348-352.

Obrein, L. and A.T. Pugsley, 1981. F3 yield response to F2 selection for gibberellic acid insensitivity in eight wheat crosses. Crop Sci., 21: 217-219.

Pattanaik, S.K., 1994. A seedling physiotaxometric study bearing on adaptation in ragi. M.Sc. Thesis, Orissa University of Agriculture and Technology.

Poehlman, J.M. and D. Borthakur, 1969. Breeding Asian Field Crops. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp: 285-288.

Portis, E., C. Comino, A. Lenzi, P. Lombardi, R. Tesi and S. Lanteri, 2004. Amplified fragment length polymorphism for variety identification and genetic diversity assessment in Oleander (*Nerium oleander* L.). Euphytica, 136: 125-137.

Pretova, A., 1980. Effect of streptomycin on growth of immature flax (*Linum usitatissimum*) embryos. Biologia, 35: 413-416.

Qi, X., J.N. Kimatu, Z. Li, L. Jiang, Y. Cui and B. Liu, 2010. Heterotic analysis using AFLP markers reveals moderate correlations between specific combining ability and genetic distance in maize inbred lines. Afr. J. Biotechnol., 9: 1568-1572.

Rath, D.M., 1977. Studies of adaptation in wheat and barley. M.Sc. Thesis, Orissa University of Agriculture and Technology.

Samantarai, B. and S.K. Sinha, 1955. Relationship of certain hormonal and nutritional factors to the region of root emergence in isolated leaves. J. Indian Bot. Soc., 34: 107-111.

Saric-Kundalic, B., S. Fialova, C. Dobes, S. Olzant and D. Tekel'ova *et al*., 2009. Multivariate numerical taxonomy of *Mentha* species, hybrids, varieties and cultivars. Sci. Pharm., 77: 851-876.

Satpathi, M., 1991. Studies on the possible use of streptomycin as an aid to germplasm evaluation in crop plants. Ph.D. Thesis, Utkal University.

Saulescu, N.N. and W.E. Kronstad, 1998. Genotypic differences in leaf chlorophyll loss during dark induced senescence in seedlings of winter wheat. (*Triticum aestivum* L.). Rom Agric. Res.

Saulescu, N.N., L. Ciocazan and C. Lazar, 1998. Genotypic differences in dark-induced senescence are correlated with field stay green scores in maize (*Zea mays* L.). Rom Agric. Res.

Saulescu, N.N., G. Ittu and P. Mustatea, 2001. Dark induced senescence as a tool in breeding wheat for optimum senescence pattern. Euphytica, 119: 205-209.

Saulescu, N.N. and P. Mustatea, 2002. Dark induced senescence in seedlings is correlated with flag leaf senescence in the field. Annu. Wheat Newslett., 45: 1-3.

Shuaib, M., Z. Alam, A., Zahir, A. Waqar, A. Taufiq and K. Ikhtiar, 2007. Characterization of wheat varieties by seed storage-protein electrophoresis. Afr. J. Biotechnol., 6: 497-500.

Singh, B. and P.R. Nanda, 1997. Prediction of adaptation pattern of some mungbean genotypes based on streptomycin response. Environ. Ecol., 15: 559-561.

Singh, B., S. Das and S.K. Sinha, 2008. Laboratory evaluation of adaptation through seedling response to chemicals in mungbean - A novel approach. Proceedings of the 5th International Food Legumes Research Conference (IFLRC V) and 7th European Conference on Grain Legumes (AEP VII), April 26-30, Antalya, Turkey.

Singh, B. and S.K. Sinha, 2010. Enhanced sensitivity to mutagens by pre-soaking- A taxometric study using M1 seedling traits in finger millet. Indian J. Agric. Res., 44: 38-42.

Sinha, S.K., 1964. Leaf culture as a tool in potato breeding. Indian Potato. J., 6: 49-53.

Sinha, S.K. and M.B. Satpathi, 1977. Streptomycin induced variation in albinism in inbred maize. Sci. Cult., 43: 557-558.

Sinha, S.K. and M.N. Swain, 1978. Chemically aided selection in two EMS derived population of ragi. Proceedings of the 65th Session of Indian Science Congress, Jan. 3-7, Ahmedabad, pp: 205-205.

Sinha, S.K. and M.B. Satpathi, 1979. An evidence for relationship between streptomycin sensitivity of plastids and high yield potential in rice. Sci. Cult., 45: 373-374.

Sinha, S.K. and B.K. Mohapatra, 1994. Preferential Induction of micro-mutations in cereal improvement. Plant Sci. Res., 16: 1-4.

Sinha, S.K., N. Prusty and S. Das, 1996. Laboratory evaluation of adaptation on ragi (*Eleusine coracana* Gaerotn). Numerical classification of seedling response to streptomycin. Plant Sci. Res., 18: 57-61.

Sip, V., M. Skorpik and J. Taborska, 1986. Method of testing response to GA application for the detection of dwarfing genes in wheat. Genetika Slechteni, 22: 133-141.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy: The Principles and Practices of Numerical Classification. W.H. Freeman and Co., San Francisco.

Spano, G., N. Di Funzo, C. Perrotta, C. Platani and G. Ronga *et al*., 2003. Physiological characterization of Stay green mutants in durum wheat. J. Exp. Bot., 54: 1415-1420.

Spivakov, N.S. and E.L. Klimashevski, 1989. Sorghum mutants with altered characteristics of root nutrition as genetic resources for directed breeding of phosphorous-effective forms. Doklady Vsesoyuznoi Ordena Leninai Ordena, 10: 19-21.

Syme, J.R. and J.P. Thompson, 1986. Stripe rust reactions of Australian wheats. Euphytica, 35: 593-602.

Szlachciak, J. and A. Boron, 2003. A numerical taxonomic study of several *Cobitis* species (Pisces, Cobitidae) based on their cytogenetic features. Folia Biol., 51: 7-11.

Von Euler, M., 1947. Einfluss des streptomycin and dischloro phyllibildung. Kem. Arb., 9: 1-3.

Zubko, M.K. and A. Dey, 1998. Stable albinism induced without mutagenesis: A model for ribosome free plastid inheritance. Plant J., 15: 265-268.