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Crop germplasm and wild relatives: a source of novel variation for crop improvement

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Plant genetic resources (germplasm) are the most valuable among the natural resources, as they provide building blocks for developing improved cultivars. The continuing need for improved crops cultivars to meet challenge of climate change, changes in pest scenario and changing consumer demands creates a constant need for germplasm. However, the crop germplasm pool is shrinking mainly due to negative actions of humans. Germplasm are in danger of genetic erosion, and/or local extinction because of habitat loss, alteration resulting from agriculture, loss of forest due to urbanization, pollution, invasion of exotic species, and their over exploitation. Rapid loss of plant biodiversity during the twentieth century and realization of its importance led to the collection and conservation of over 6.1 million accessions in over 1400 genebanks globally. International Agricultural Research Centers maintain over 600,000 accessions. ICRISAT genebank conserves 118,882 accessions of chickpea, pigeonpea, groundnut, sorghum, pearl-, finger, foxtail, barnyard, proso, little, and kodo millets from 144 countries (Table 1). Knowledge and management of genetic resources are critical for crop improvement programs.

Low use of germplasm

The large germplasm collections in the genebanks have not been used adequately in international and national crop improvement programs. For example, very few of the >20,000 chickpea accessions conserved in the genebank (<1%) have been utilized in cultivar development at ICRISAT (Upadhyaya et al., 2006a). Similarly, in the national programs, the germplasm lines used in breeding programs are very limited. In China, the introduced germplasm and wild relatives have seldom been used in groundnut improvement. In USA, the cultivar 'Dixie Giant' was a germplasm source in all pedigrees of runner type groundnut and 'Small White Spanish-1' accounts for >90% pedigrees of all released cultivars. Similar situation has been reported in many crops as most plant breeders prefer to work with their own breeding lines, rather than exotic germplasm materials. Not only the limited use of germplasm is a worrisome issue, the large-scale deployment of cultivars with common genetic background complicates the whole situation even more. Sometimes, even if the number of cultivars is more, the degree of genetic diversity between them is very low and can lead to epidemics similar to the southern corn leaf blight in the USA (resulting in huge economic loss) and late blight of potato (that wiped out the potato crop resulting in famine in Europe) due to narrow genetic base of crop cultivars. One of the main reasons for low use of germplasm is the lack of information on economic traits that display high genotype x environment interactions and require multilocal replicated evaluations to identify parents for use in crop improvement

programs. Development of core collection (10% of entire collection) representing diversity of entire collection has been suggested to overcome this.

Strategy to enhance the use of germplasm

Development of core collection

A pre-requisite before developing a core collection is to assess and characterize the diversity in the germplasm collections. At ICRISAT, we have assessed the patterns of diversity in chickpea, groundnut, pigeonpea, sorghum, pearl millet, finger millet and foxtail millet germplasm collections using data on qualitative and quantitative traits. Core collections have been developed using the information on regional patterns of diversity (Table 2).

Mini core collection

Due to its reduced size compared to entire collection, core collection is useful for scientists to identify accessions with beneficial traits for use in crop improvement programs. However, it soon became evident that developing core collections will not solve the problem of low use of germplasm, as even the size of core collection would be unwieldy for convenient exploitation by the breeders and other crop improvement scientists. This was particularly true in the crops where entire collection is too large (several thousands). To overcome this, Upadhyaya and Ortiz (2001) proposed the "mini core collection" concept using a two-stage strategy. The first stage involves developing a representative core collection (about 10%) from the entire collection using all the available information on origin, geographical distribution, and characterization and evaluation data of accessions. The second stage involves evaluation of the core collection for various morphological, agronomic, and quality traits, and then selecting a further subset of about 10% accessions from the core collection. Thus the mini core collection contains 10% of the core or ~ 1% of entire collection, but represents the diversity of the entire collection. At ICRISAT, mini core collections of chickpea, groundnut, pigeonpea, sorghum, and pearl millet have been established (Table 2). Mini core collections have been extensively used to identify trait specific germplasm for use in crop improvement programs.

Identifying trait specific germplasm using core/mini core approaches

The core and mini core collections of chickpea, groundnut, and pigeonpea have been evaluated extensively to identify new sources for important biotic and abiotic stresses and for agronomic traits. In chickpea, we have identified sources for early maturity (28) (Upadhyaya *et al.*, 2007b), large-seeded kabuli type (16), high yield and other agronomic traits (39) (Upadhyaya *et al.*, 2007a), and resistance/tolerance to drought (18) (Kashiwagi *et al.*, 2005), salinity (12) (Vadez *et al.*, 2007), high temperature (5), helicoverpa pod borer (5), fusarium wilt (67), botrytis gray mold (55), ascochyta blight (3), dry root rot (6), and multiple resistance (31 accessions) (Pande *et al.*, 2006). Similarly in groundnut scientists have identified sources for early-maturity (21)

(Upadhyaya et al 2006b), high yield, large seed size and high shelling percentage (60) (Upadhyaya et al, 2005); resistance/tolerance to drought (18) (Upadhyaya, 2005), salinity (12); low temperature at germination (158) (Upadhyaya et al, 2008b), aflatoxin (5), rosette (3), and early leaf spot (1); in pigeonpea for early maturity (20), high yield, large seed size and high harvest index (54), resistant/tolerant to salinity (16) (Srivastava et al, 2006), sterility mosaic (11), wilt (4), Phytophthora blight (8), and two multiple resistant germplasm; in sorghum 28 germplasm with sweet stalk and 10 resistant to grain mold; 25 germplasm each in finger millet and foxtail millet for high grain yield and early maturity; several accessions for early maturity, high grain yield, and large seed size in pearl millet. The mini core collections were also evaluated by NARS partners. A total of 88 sets of core and mini core collections have been provided to scientist in 19 countries of Africa, Asia, Americas, and Europe. NARS partners identified several parents for use in breeding in India, China, Vietnam, Thailand, Mali, Niger, and UAE

Genotyping germplasm collections:

The revolution in molecular biology, bioinformatics, and information technology has provided the scientific community with tremendous opportunities for solving some of the world's most serious agricultural and food security issues. The Generation Challenge programme (GCP) on "Unlocking Genetic Diversity in Crops for the Resource-Poor (www.generationcp.org)" is enhancing research on this area. ICRISAT in collaboration with partners such as ICARDA, Syria; CIRAD, France; EMBRAPA, Brazil; and CAAS, China has developed the composite collections of sorghum, pearl millet, chickpea, pigeonpea, groundnut, finger millet, and foxtail millet (500 – 3500 accessions) (Table 2). The composite collections include core and mini core collections and have been genotyped using 20 to 50 SSR markers to study genetic diversity, population structure, and to establish reference sets of 200–400 genetically diverse accessions (Table 2). The composite collections were also characterized for morpho-agronomic traits at ICRISAT Center, Patancheru, India. Reference sets based on SSR markers, qualitative traits, quantitative traits, and their combinations were formed and compared for allelic richness and diversity. In chickpea, for example 48 SSR-based reference set captured 78.1% alleles of composite collection (1683 alleles) compared to 73.5% of alleles in the reference set based on 7 qualitative traits. The reference set based on both SSR and qualitative traits captured 80.5% (1354 alleles) of composite collection (Upadhyaya et al., 2008a). Similarly, in groundnut the SSR-based reference set captured 95.1% alleles (466) of composite collection (490) compared to 93.3% of alleles (457) in the reference based on 14 qualitative traits. The reference set based on both SSR and qualitative traits captured 95.9% (470) alleles of composite collection. This demonstrated that both SSR and qualitative traits were equally efficient in capturing the allelic richness in reference sets.

Use of novel alleles from wild relatives for enhancement of agronomic traits

Potential of wild relatives to enhance the resistances to biotic and abiotic stresses in cultivated species is well recognized in several crops. The wild relatives are considered agronomically poor and have seldom been considered for enhancing agronomic traits

such as early-maturity, seed size, seed yield, and harvest index. Our research at ICRISAT has indicated that the novel genes of wild relatives of chickpea, groundnut, and pigeonpea can enhance the agronomic traits of cultivated species. For instance, using two accessions of *Cicer reticulatum* (110-113 days to 50% flowering, 143-150 days to maturity, 12-16 g 100-seed weight, 2-5 g plant yield, 5.7 to 16.1% harvest index) as a parent, we have selected several progenies which took 8-21 days less to flower and 6-33 days less to mature, produced 20-103 % larger seeds, 97-217 % greater seed yield, and 6-68 % higher harvest index than the respective cultivated parents. Similarly in groundnut using an amphidiploid with very low 100-seed weight (5-8 g) and poor pod yield (2-5 g plant⁻¹), progenies with 60-80% higher 100-seed weight and 150-250% more pod yields were identified. This demonstrated that the novel alleles of wild relatives, that were considered to be lost in evolution to cultivated types, could be used to enhance the important agronomic traits in legumes.

Conclusions

Germplasm is basic to crop improvement programs for sustainable agriculture. Trait specific, genetically diverse parents are basic requirements of plant breeders for trait enhancement. Agronomically superior lines are preferable for use in breeding due to the scope for exploitation of additive genetic variance. This would reduce role of unpredictable epistatic and dominance deviation variances. Our strategic research on core, mini-core, composite collections, identifying genetically diverse trait specific germplasm and unlocking potential of wild relatives in improving agronomic traits would be helpful in achieving quantum jump and providing impetus to breeding programs in developing cultivars with a broad genetic base.

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Allotropy in rice can be used as a method for biological control of weeds in rice cropping. We have focused on detecting QTL for allelopathic effect and identifying chemical compounds associated with allelopathic effect in rice. Firstly, we developed a reproducible bioassay to assess the allelopathic effect of rice. We analyzed QTL conferring allelopathic effect of rice using F2 plants of the cross between P312777 (allelopathic) and Rexmont (non-allelopathic) and RFLP markers mapped on 12 rice chromosomes. Seven QTLs for allelopathic effect were identified on chromosomes 1, 3, 5, 5, 7, 11 and 12. Major QTLs are on the chromosome 5, 6 and 7, explaining 13–16% of total phenotypic variation. A single QTL on chromosome 7 showed a positive effect on allelopathic effect without QTLs on chromosomes 5 and 6. QTL on chromosome 7 is responsible for inhibition of root growth and necrosis on root tips of lettuce. We also identified candidate compounds associated with allelopathic effect of rice using HPLC. Among 20 phenolic compounds identified in rice, at least 7 phenolic compounds might play primary roles in allelopathic effect of rice. QTL analysis and chemical identification will be discussed in this report.

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CS2-S1, 05 (16:20-16:40)

Developing the Wheat Phenome Atlas: Integrating 25 years of International wheat Phenotypic data with Genome Analysis

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The Wheat Phenome Atlas Project will develop an integrated diagrammatic representation of the regions of the wheat genome that influence heritable phenotypic variation for agronomic, pests and disease resistance and quality traits. Developing the analytical tools to deal with data sets of 15,000 lines x 40 years x 80 traits x 100 locations x 2,000 DNA data points (almost 10 trillion data points) will involve the development of new and powerful bioinformatic tools and webpage visualization software. With the consortium we are developing, we will develop a Wheat Phenome Atlas Toolbox which will be freely available to researchers worldwide. The toolbox will enable researchers to develop other crop phenome atlases when appropriate data become available. It will allow for identification of elite gene blocks leg for rust resistance, the molecular tools for rapidly introducing those elite gene blocks into new cultivars, and the prediction of cultivar performance in a range of environments, including future environments predicted by climate change models. As a pilot study we are analysing data from the Elite Spring Wheat Yield Trials (ESWYT). We are integrating the phenotypic data from 25 years of ESWYT, a total of 675 cultivars, with DAR data. Connecting these data islands will enable us to find specific genetic regions associated with 7 traits including resistance to 3 rusts (leaf, stem and stripe), grain yield, seed size, plant height and days to heading.

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CS2-S1, 06 (16:40-17:00)

Core Collection for Enhance Use and Sustainable Conservation of Plant Genetic Resources: Case Studies Using Heuristic Approach for Indian Small Millets Collections

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In the context of limited resources available, increasing size of germplasm collections in most genebanks limits their accessibility for use in crop breeding and quality of their management. Therefore, it has been proposed that a limited set of accessions be selected containing as much genetic diversity as possible to offer a good starting point when searching for new traits, and could be used for in-depth evaluation, thus increasing the knowledge of the entire collection. Frankel (1984) introduced this concept, calling it a "core collection". Frankel and Brown (1984) and Brown (1989a, 1999a) further developed the concept. Many genebank curators around the world have now been working with core collection. As a result, the concept evolved from a theoretical idea into widely applied methods with many variations and has resulted in a wide variety of methodologies for identifying core entries. Basically, the procedure of creating a core collection is very simple and a random selection from a collection might be considered a core collection. But the selection of a core collection to ensure representative diversity is some what more complicated. A most common approach being use for developing core collections

use the following steps: (i) defining the material that should be represented in the core, (ii) divide the material into groups, which should be as genetically distinct as possible, (iii) choice of number of entries per group, which will depend on the overall size of the core collection, and (iv) choice of entries from each group that are to be included in the core. Over the years, tremendous progress has been achieved using different methodologies, including stratified random sampling, and these methodologies have been successfully applied to develop core collections for various genebanks. More recently, the staffs of the Rural Development Administration (RDA) has used the advanced M strategy with a heuristic search for establishing core sets and accordingly a programme known as "PowerCore" has been developed and made available to users (<http://genebank.rda.go.kr/powercore/>). The programme support development of core-sets by reducing the redundancy of useful alleles and thus enhancing their richness. Output of the "PowerCore" has been validated using some case studies and the programme effectively simplifies the generation process of core-sets while significantly cutting down the number of core entries, maintaining 100% of the diversity. PowerCore is applicable to various types of genomic data including single nucleotide polymorphisms (Kyu-Wan Kim et al. 2007). In the present study, "PowerCore" has been used to develop core sets of six Indian small millet collections viz., Barnyard (*Echinochloa frumentacea*), Finger (*Echinochloa coracana*), Foxtail (*Setaria italica*), Kodo (*Paspalum scrobiculatum*), Little (*Panicum sumatrense*), and Proso or common millet (*Panicum miliaceum*). The number of accession used for developing the core sets were: 729, 3924, 1478, 1038, 922 and 672 for Barnyard, Finger, Foxtail, Kodo, Little, and Proso millet, respectively. The validation of core sets using "PowerCore" had also been compared with the core developed using the stratified methodology, as suggested above. It has been observed that the core sets identified using "PowerCore" are small in size with greater diversity captured compared to traditional clustering methods used for all the six millets collections and the results has been discussed in this presentation.

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CS2-S1, 07 (17:00-17:20)

In Situ Conservation and Characterization of Genetic Resources in the Genus *Vigna* Sub-Genus *Ceratotropis* in Thailand

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This project is part of the conservation and utilization of field crops genetic resources research project of the Thai DOA and partly funded by the FAO-APR. The prime aim of the project was 1) to make an inventory of known locations where *Vigna* species can be found, 2) to undertake field visits to validate the findings and make recommendation to conserve the populations found in those locations for in situ conservation of *Vigna* species and 3) to raise public awareness of the importance of in situ conservation and sustainable use of *Vigna* species. Nine surveys were carried out between December 2005 and May 2005 and covered 12 provinces of Thailand. Found locations were recorded by a GPS. Sixty-five sites from previous records were searched. It was found that 41 sites had disappeared and only 24 sites were found. Sixty-six samples, of plant for herbarium specimens; of seed for ex situ regeneration and of rhizome for beneficial microorganism analysis, were collected. Wild *Vigna* in several sites had disappeared due, perhaps, to threats imposed to the conservation sites, both abiotic or biotic ones. Abiotic threats were such as slash-and-burn, road expansion, urbanization, or herbicide application. Biotic threats were such as disease, insect pests, nematode or leoparchy effect. Maps of wild *Vigna* species distribution as well as their in situ sites were also displayed. Attempt, when possible, was made to relieve threats in order to sustain these in situ conservation sites. This case study should be monitored at least every 5 years.

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CS2-S1, 08 (17:20-17:40)

Allelic Richness and Diversity in Chickpea (*Cicer Arietinum* L.)

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Chickpea is the fourth largest grain legume crop globally. A composite collection of 3000 accessions was formed and genotyped using 50 SSR markers. The accessions were also field evaluated for seven qualitative traits. Analysis of 48 SSR markers data on 2915 accessions detected 1683 alleles, of which 935 were rare and 749 common. Gene diversity varied from 0.533 to 0.975. Kabuli chickpea as a group were genetically more diverse than other seed types. Several group-specific unique alleles were detected: 104 in Kabuli, 257 in desi, and 68 in wild Cicer; 114 each in West Asia and Mediterranean, 117 in South and South East Asia, and 10 in African region accessions. A genotype-based reference set captured 1315 alleles compared to 1237 alleles in the reference set based on qualitative traits or 1354 alleles based on SSRs and qualitative traits data. The relative usefulness of these reference sets in chickpea breeding and genomics studies are being further investigated. Corresponding author: Hari Upadhyaya, hupadhyaya@cgiar.org

Local Aromatic Rice Cultivars of North Himalayas: present status and future strategies for their sustainable improvement Ghulam Ahmad Parraay, Asif Bashir Shikari, Manzoor A Gani and N. R. Sofi Sher-Akash University of Agricultural Sciences and Technology of Kashmir Rice Research and Regional Station Khudwani The expansion of area under irrigated rice and the efforts made by the rice breeders during the past 5-6 decades have led to the development of improved and high yielding rice varieties that have kept a pace with the rapidly growing human population, and has guaranteed a food security for the people. However, cultivation of lower high yielding varieties, specific to a particular agro-ecological situation, has brought about the danger of genetic vulnerability as genetic uniformity of improved rice varieties replaced the diversity of indigenously adapted rice cultivars that were under cultivation over centuries. Kashmir valley has been known for large number of high quality indigenous rice cultivars for their milled rice grain characteristics and better cooking quality, with a few possessing aroma. These local cultivars are, by and large tall, photosensitive, poor yielders and susceptible to paddy blast. As in other parts of the country and rice growing belts on the globe, spread and adoption of high yielding rice varieties in the Kashmir during the past 3-4 decades also led to the genetic erosion of these indigenous high quality rice cultivars. During this transition phase many of these rice cultivars/ecotypes have been lost. Among the most prominent aromatic indigenous rice cultivars of the Kashmir, Mushk Bujji stands at the top followed by Kamad. These cultivars are now being grown over small fragmented areas in the Sagar and Pangpora belts, and Soat Shali village of district Anantnag and Baramulla belt of the district Budgam. Both these cultivars are short grained and bold seeded with excellent cooking quality. These cultivars emit aroma in the field, at harvesting, in storage, during milling and polishing, cooking and while eating. The consumption of these aromatic rice has now been confined to special occasions and marriage festivals of the rich people of the valley. This is primarily because of the very high sale price of milled rice of these cultivars. Salient morphological, yield and grain/cooking quality attributes of these two cultivars are given in the Table-1. Table-1: Salient morphological, yield and quality traits of aromatic rice cultivars of Kashmir. Salient features Aromatic rice cultivars High yielding varieties Mushk Bujji Kamad. Jilum (check)* K-332 (check)* A. Morphological: i. Plant height (cm) 105.0 (95.0-120.0) 110.0 (98.0-125.0) 110.0 (102.0-120.0) 80.0 (72.0-88.0) ii. Days taken to maturity 118.0 (115.0-125.0) 122.0 (118.0-128.0) 133.0 (130.0-140.0) 139.0 (135.0-140.0) B. Yield components i. Average grains/ panicle 130.0 126.0 210.0 155.0 ii. Average 100-grain weight (g) 30.0 29.0 24.0 23.0 iii. Harvest index (%) 42.0 42.0 47.0 45.0 iv. Grain yield (t/ha) 15.20 13.18 45-55 34-38 C. Quality parameters v. Av. Hulling (%) 62.0 61.0 75.0 75.0 vi. Av. Milling (%) 75.0 75.0 72.0 71.0 ix. Av. Head rice recovery (%) 68.0 58.0 62.0 64.0 x. Av. Kernel LS ratio 2.02 (short grain) 2.06 (short grain) 2.58 (medium grain) 2.08 (short grain) xi. Av. Kernel elongation on cooking (mm) 10.8 11.1 12.3 10.8 xii. Av. Amylose content 24.7 24.8 27.9 24.5 xiii. Av. Gelatinization temperature Intermediate Intermediate Intermediate xiv. Aroma score 3 (highly scented) 3 (highly scented) 1 (non scented) 1 (non scented) * Jilum and K-332 are standard check varieties (high yielding) for valley basin (1550-1700 m amsl) and high altitude area (>1750 m amsl) of Kashmir. Most of the perennials resources of these local adapted quality rice cultivars are presently maintained, in pure form, at the Regional Rice and Research Station, Khudwani. The seeds are renewed every year and breeders seed to the extent of 40-50 kg of both these aromatic rice cultivars can be immediately multiplied for distribution among the interested farmers once the indent is received properly from the Agriculture Department. Grain yield is the most important parameter to farmers while the crop is in the field, but when the final product of the crop i.e. milled rice reaches the market, quality becomes the key determinant of its sale price. Consumer's concern is basically to get rice of good cooking (head rice, colour, amylose content, gel consistency, kernel elongation on cooking, softness, etc.) and eating (taste and aroma) qualities. Best cooking and eating qualities are determined by physico-chemical properties of milled rice. An analysis of benefit-cost ratio of the cultivation of aromatic rice vis-a-vis the standard high yielding varieties is presented in the Table-2. Table-2. Benefit-cost ratio of cultivation of aromatic rice. Corresponding author: Ghulam Parraay, ahmadparraay@yahoo.co.in

CS2-S1, P4 An Assessment of Genetic Diversity in Cultivated Tea (*Camellia Sinensis* L.)

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Tea (*Camellia sinensis* L.) is the most important beverage crop in Iran and the genetic base of tea plantations originated from three Jats introduced from India in 1930. For determination of genetic diversity among 30 cultivated tea clones including of national selections and introduced genotypes from Sri-Lanka, Russia, India and Japan, they were evaluated using 45 RAPD markers. A total of 98 bands were produced from 16 RAPD markers that were consisted of 79 polymorphic bands and 17 monomorphic bands. Polymorphic band percentage was estimated as 82.3

Poster Session

CS2-S1, P1 Evaluation of Iranian Garlic (*Allium sativum* L.) Genotypes Using Multivariate Analysis Method Based on Morphological Characteristics

Bahman Zahedi¹
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Genetic evaluation of 38 *Allium sativum* L. genotypes collected from different parts of Iran was carried out using morphological characteristics. Measuring of characteristics of garlic genotypes were performed based on IPGRI descriptor. Analysis of variance revealed high level of variability among all genotypes. Factor analysis was used to determine the number of main factors. The results showed, the most built characters composing the main factors. Effective characters categorized in seven factors contributing 81.84 % of total variance. For each factor eigen value of more than 0.5 was used as significant eigen value. Cluster analysis was performed using seven factors. Garlic genotypes in distance of 10 [similarity coefficient] were divided into four main clusters. This study showed that multivariate analysis is a useful method for discrimination of garlic genotypes. Keywords: Garlic, morphological characteristics, multivariate analysis, factor analysis, cluster analysis
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CS2-S1, P2 Effects of Different Plant Growth Regulators and Potting Mixes on Micropropagation and Minutuberization of Potato Plantlets

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For this study, Agria and Marfona cultivars of potato (*Solanum tuberosum* L.) were used. After thermotherapy and meristem isolation of cultivars, their meristems were placed on filter paper bridge in liquid medium. The plantlets resulted from meristems were micropropagated in solid medium by using single node cuttings culture method. Plantlets were grown at 25°C and 16 h photoperiod with 2000 lux light intensity for one month. Then the effects of different combinations of NAA (auxin) and BAP (cytokinin) hormones (each in four concentrations: 0, 0.5, 1 and 1.5 mg/l) were investigated on rooting and growth of potato single nodes. Modified solid (MS) medium with GA3 (0.25 mg/l) and calcium pantothenate (2 mg/l) were applied in this stage. Results showed that there are significant differences between Agria and Marfona cultivars. The best medium for rooting and shooting was modified solid (MS) medium without NAA and BAP. Application of BAP and NAA decreased shooting and rooting of single nodes in medium culture. Also, after plantlets micropropagation in selective medium (control), the effects of four potting mixes on plantlets morphological traits and minutuber production were studied in greenhouse. Results showed that: there are significant differences between different potting mixes. Peat moss/sand (4:1) was the best potting mix for plantlets growth and minutuber production.
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CS2-S1, P3 Local Aromatic Rice Cultivars of North Himalayas - Present Status and Future Strategies for Their Sustainable Improvement

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