teins. It is interesting to assume that this should also be true *in vivo* during protein synthesis because, after synthesis, the folding of the polypeptide remains sensitive to antibiotics for several minutes due to its association with the domain V of 50S subunit[6]. Our aim is to do similar experiments in *in vitro* translation system in the presence of release factors, elongation factor EFG and the ribosome recycling factor.

1. Nakamura, Y., Ito, K. and Isakson, L. A., *Cell*, 1996, **87**, 147–150.
2. Janosi, L. *et al.*, *EMBO J.*, 1998, **17**, 1141–1151.
3. Atarashi, K. and Kaji, A., *J. Bacteriol.*, 2000, **182**, 6154–6160.
4. Das, B., Chattopadhyay, S. and DasGupta, C., *Biochem. Biophys. Res. Commun.*, 1992, **183**, 774–780.
5. Das, B., Chattopadhyay, S., Bera, A. K. and DasGupta, C., *Eur. J. Biochem.*, 1996, **235**, 613–621.
6. Chattopadhyay, S., Pal, S., Pal, D., Sarkar, D., Chandra, S. and DasGupta, C., *Biochim. Biophys. Acta*, 1998, **1429**, 293–298.
7. Chattopadhyay, S., Das, B. and DasGupta, C., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 8284–8287.
8. Pal, S., Chandra, S., Chowdhury, S., Sarkar, D., Ghosh, A. N. and DasGupta, C., *J. Biol. Chem.*, 1999, **274**, 32771–32777.
9. Chowdhury, S., Pal, S., Ghosh, J. and DasGupta, C., *Nucleic Acids Res.*, 2002, **30**, 1278–1285.
10. Sanyal, S. C., Pal, S., Chaudhuri, S. and DasGupta, C., *Nucleic Acids Res.*, 2002, **30**, 2390–2397.
11. Cata, J. H., Yusupov, M. M., Yusupova, G. Z., Earnest, T. N. and Noller, H. F., *Science*, 1999, **285**, 2095–2104.
12. Nissen, P., Hansen, J., Ban, N., Moore, P. B. and Steitz, T. A., *Science*, 2000, **289**, 920–930.
13. Ban, N., Nissen, P., Hansen, J., Moore, P. B. and Steitz, T. A., *Science*, 2000, **289**, 905–919.
14. Culver, G. M., Cate, J. H., Yusupova, G. Z., Yusupova, M. M. and Noller, H. F., *Science*, 1999, **285**, 2133–2136.
15. Teter, S. A. *et al.*, *Cell*, 1999, **97**, 755–765.
16. Choi, K. M. and Brimacombe, R., *Nucleic Acids Res.*, 1998, **26**, 887–895.
17. Joseph, S. and Noller, H. F., *EMBO J.*, 1996, **15**, 910–916.
18. Karimi, R., Pavlov, M. Y., Buckingham, R. H. and Ehrenberg, M., *Mol. Cell*, 1999, **3**, 601–609.
19. Hirashima, A. and Kaji, A., *J. Mol. Biol.*, 1972, **65**, 43–48.
20. Subramaniun, A. R. and Davis, B. D., *J. Mol. Biol.*, 1973, **74**, 45–56.
21. Pavlov, M. Y., Fresitroffer, D. V., Heurgue-Hamard, V., Buckingham, R. H. and Ehrenberg, M., *J. Mol. Biol.*, 1997, **273**, 389–401.
22. Selmer, M., Al-Karadaghi, S., Hirokawa, G., Kaji, A. and Liljas, A., *Science*, 1999, **286**, 2349–2352.

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STMS-based DNA fingerprints of the new plant type wheat lines

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New plant type wheat lines have been developed at the Indian Agricultural Research Institute, New Delhi, by combining some of the negatively correlated yield contributing traits such as more grains per panicle, larger grain size and optimum tillers per plant through inter-varietal hybridization followed by a modified-bulk pedigree method of selection. Some of these lines have been found to give 15–22% higher yield in experimental plots than the most popular national check varieties being cultivated in the north-western part of India and thus constitute a valuable source of genetic variation for breaking yield barriers in wheat. With the objective of precise differentiation and identification of new plant type wheat lines, 33 sequence tagged microsatellite site markers, which are genetically located on different chromosomes of wheat genome, were employed. These markers in combination differentiated the new lines from 137 wheat varieties released for commercial cultivation in India. Fourteen markers detected polymorphism among the thirteen genotypes included in this study, of which eight markers in combination differentiated the seven new wheat lines from each other as well as from their parents. Inheritance of parent-specific alleles revealed the direction of selection during breeding. Grouping of the lines based on cluster analysis reflected the nature of their relationship with the parent varieties. A graphic presentation of the genetic constitution of the new plant type lines was developed, which can be used as bar-coded molecular tags for identification of the respective seed samples.

THE Green Revolution in India in the 1960s was primarily due to the introduction and large-scale cultivation of semi-dwarf wheat varieties developed at CIMMYT, Mexico. The new plant architecture replacing the traditional tall phenotypes was responsible for a quantum jump in wheat yield. To bring further significant improvement in the yield potential, attempts are being made to modify the wheat plant by breaking some of the negative correlations among the yield contributing traits, namely number of productive tillers per plant, number of grains per panicle and grain size. At the Indian Agricultural Research Institute (IARI), New Delhi, strategic research during last seven years has led to the development of a new plant type by combining intermediate to high tiller

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number (≥ 375 per m^2), more grains per spike (95–110), higher grain weight (> 50 g per 1000 grains), higher biomass, dark green and broad leaves, thick stem, 120–135 days maturity duration, 85–100 cm plant height and resistance to leaf and stem rust diseases. Some of these lines have given 15–22% higher yield than the leading commercial varieties including PBW343, HD2329 and UP2328 in experimental plots[1]. These lines have been evaluated at the IARI, New Delhi and will soon be tested in National Trials. These are also being used in new wheat breeding programmes as donors of desirable genes[2]. Unambiguous identification of these genotypes is essential for protecting the lines from unauthorized use and testing purity of their seed samples.

Molecular markers assay genetic variation at the nucleotide sequence level. They follow simple Mendelian pattern of inheritance and remain unaffected by the growth and environment, thus permitting precise identification of genotypes. In particular, markers based on polymerase chain reaction (PCR) such as sequence-tagged microsatellite sites (STMS) are highly reproducible, allow automation and therefore, facilitate analysis of a large number of samples in a short time. In STMS, a pair of primers flanking simple sequence repeat sequences (microsatellites) specifically amplifies the target sequence, revealing a high degree of allelic variation[3–6]. Moreover, microsatellites being abundant in plant genomes, yield STMS markers distributed all over the genome. In wheat, STMS markers detecting variation at defined genetic loci have been used to construct a linkage map[7]. These markers can be judiciously used to reveal variation in unlinked and thus non-overlapping genomic regions, thereby providing an unbiased estimate of genetic relatedness among individuals. In the present study, STMS markers were used to establish DNA fingerprint-based identity of desirable new plant type wheat genotypes and determine their relationship with the parental lines.

Seven new plant type wheat genotypes, namely DL1266-1, DL1266-2, DL1266-5, DL1266-6, DL1266-10, DL1266-16 and DL1266-17 were characterized along with their parents. These lines were developed from the cross of SFW (a local germplasm called Sirsa Farm Wheat) with Vaishali (KAL*4//TR380.27*4/3AG/3/HD2281) by a modified-bulk pedigree method[1]. Two breeding lines, namely DL1337-1 and DL1396-11, having pedigree different from the new plant types as well as two commercial varieties, PBW343 and HD2329, were also included in the study for comparison. These lines possess significantly higher number of grains/spike and 1000-grain weight along with an optimum number of tillers/m^2 (Table 1). The line DL1266-5 has been found in replicated trials to have the most desirable combination of these yield-contributing traits that has resulted in 22.4% more yield than the highest yielding national check variety PBW343, which occupies most part of the north-western plain zone of the country[8]. Simultaneous increase in the seed size and seed number per spike by breaking down the negative correlation between them is the most significant characteristic of these lines[1,2].

About 100 seeds of each genotype were germinated under aseptic condition. One-week-old seedlings were harvested, bulked and used for DNA isolation. DNA was extracted from fresh tissue by standard CTAB method following Mohapatra *et al.*[9], purified by RNAase treatment followed by phenol–chloroform extraction, dissolved in 10 mM Tris-HCl buffer, quantified by gel analysis using uncut λDNA as standard, diluted to 5 ng/μl and used in PCR. Thirty-three STMS markers were used for genotyping of the wheat lines. These were selected from the wheat STMS linkage map[7], taking one marker from one chromosome arm and were custom synthesized. The reaction mixture contained 10 ng of template DNA, 20 ng of each primer, 100 μM of each dNTP, 1× PCR assay buffer and 0.2 unit of Taq DNA polymerase (Bangalore Genie Pvt. Ltd., Bangalore, India) in a total volume of 10 μl. Amplification was carried out in a thermal cycler (Perkin Elmer Model 9600) with the following conditions: 94°C for 5 min followed by 35 cycles consisting of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, and finally at 72°C for 7 min. The amplified products were separated on a 3% or 3.5% Metaphor agarose gel (FMC, USA) in 1× TBE buffer, stained using gelstar dye (FMC, USA) and photographed using Polaroid photographic system.

The bands were scored and used to prepare a binary data matrix. Employing the computer package NTSYS.pc[10], Jaccard's similarity coefficients were calculated and used to establish genetic relationship among the genotypes based on unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested (SAHN) clustering.

Thirty-three mapped STMS markers, which were found informative in an assay of diversity in a set of 137

Table 1. Characteristics of new plant type wheat lines, their parents and national checks

Genotypes	No. of tillers/m^2	Grains/ spike	1000 grain weight (g)	Grain yield/ m^2 (g)
Vaishali	360	46	45	580
SFW	240	80	35	510
DL1266-1	365	80	55	620
DL1266-2	392	85	57	691
DL1266-5	385	100	56	759
DL1266-6	375	98	53	610
DL1266-10	360	100	50	630
DL1266-16	380	102	54	640
DL1266-17	370	100	47	593
DL-1337-1	285	103	43	600
DL-1396-11	299	123	40	680
PBW343	410	60	38	620
HD2329	370	61	42	560

Indian wheat cultivars released for commercial cultivation in the country in our previous study (unpublished results), differentiated the 13 genotypes analysed in this study from all those characterized earlier. These markers thus provided DNA fingerprints that would be useful for unambiguous identification of the wheat genotypes including the new plant types. Fourteen of these markers detected polymorphism among the 13 genotypes and differentiated them from each other. Polymorphism detected with four of these markers (Xgwm636-2A, Xgwm149-4B, Xgwm443-5B and Xgwm570-6A) is depicted in Figure 1. Eight markers (Xgwm357-1A, Xgwm636-2A, Xgwm493-3B, Xgwm183-3D, Xgwm149-4B, Xgwm443-5B, Xgwm182-5D and Xgwm570-6A) located on eight different wheat chromosomes individually distinguished the parental line SFW from Vaishali, and in combination, differentiated the seven new plant type lines derived from their cross from each other. Law *et al.*[11] reported that the number of polymorphic markers required for distinguishing v varieties of wheat would range from v to $2v$. Fourteen polymorphic markers differentiating the 13 genotypes and eight markers discriminating seven related sister lines from each other as observed in this study thus supports their view. In contrast, Prasad *et al.*[12] could differentiate 55 wheat genotypes using 20 STMS markers. This is because of the fact that the genotypes included in their study originated from 29 different countries representing six continents leading to detection of far more number of alleles per locus (average of 7.4) than a set of related genotypes in this study showing a maximum of two alleles per locus. In fact, differentiation of most similar varieties is an issue of concern in plant variety registration and protection, which necessitates addition of molecular data to the traditional morphological descriptors[13]. Use of different sets of varieties with varying degree of relatedness is therefore required to test the validity and applicability of the estimated number of polymorphic markers needed to be employed in wheat.

None of the wheat genotypes carried both the alleles at any of the marker loci studied, suggesting homozygosity at all the loci. DNA samples used for amplification were obtained from seedling bulks for each of the lines. Presence of intra-varietal heterogeneity would have been reflected in the form of more than one band (allele) in a single lane in varying intensities. Absence of multiple alleles indicated that the lines were also uniform in respect of all the 33 markers used. The seed samples were obtained from the concerned breeder and thus were expected to be genetically pure. The genotypic constitution at the marker loci provided the evidence for their genetic purity. It is therefore proposed that the markers that could differentiate the wheat lines from the other released varieties can be used for their purity testing. Our marker database for 137 released varieties can serve as the reference and help in identification of the admixture variety. In addition to providing the above information on varietal identity and uniformity, the co-dominant STMS markers also revealed the direction of selection with regard to the polymorphic loci in the modified-bulk pedigree method that was employed for developing the wheat lines. For instance, the lines DL1266-5 and DL1266-16 inherited six STMS alleles each, which were specific to the parental lines SFW and Vaishali respectively, whereas DL1266-6 and DL1266-10 equally shared the parent specific alleles (four each) over all the eight polymorphic loci. This observation provides a lead for further studies employing a larger set of co-dominant markers and different generations in pedigree breeding to

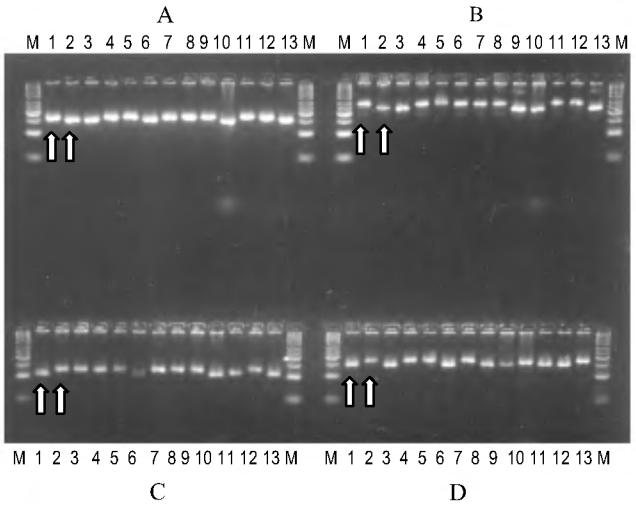

Figure 1. STMS polymorphism detected with markers Xgwm636-2A (A), Xgwm149-4B (B), Xgwm443-5B (C) and Xgwm570-6A (D). Lanes: M-50 base pair DNA ladder as size standard, 1, Vaishali; 2, SFW; 3, DL1266-1; 4, DL1266-2; 5, DL1266-5; 6, DL1266-6; 7, DL1266-10; 8, DL1266-16; 9, DL1266-17; 10, DL1337-1; 11, DL1396-11; 12, PBW343; 13, HD2329. Arrows indicate the positions of the alleles specific to the parents Vashali and SFW.

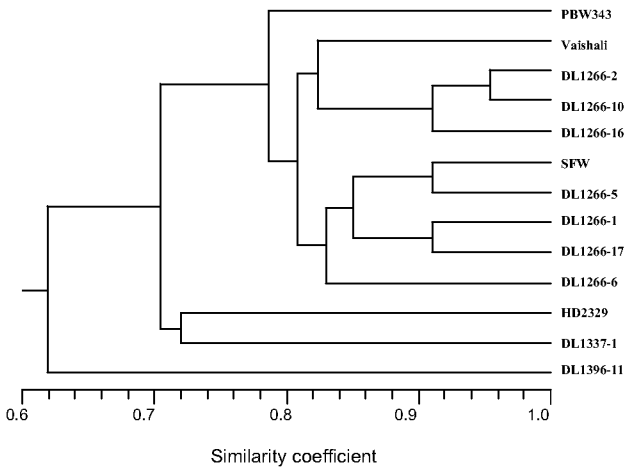

Figure 2. Dendrogram depicting the relationship among the wheat lines based on UPGMA and SAHN clustering using Jaccard's similarity coefficients.

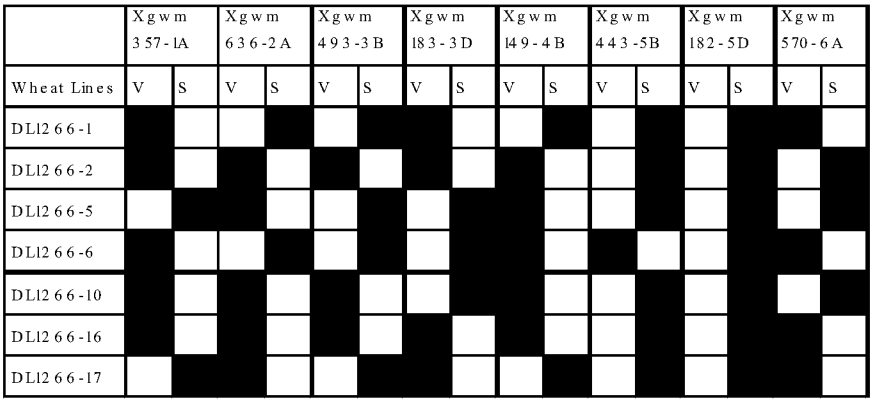

Figure 3. A graphic mode of presentation of the genotypic constitution of new plant type wheat lines based on eight polymorphic loci. The names of the lines are given in the first column. The next eight columns correspond to eight markers and the two sub-columns under each marker column correspond to allelic states of the parents Vaishali (V) and SFW (S), respectively. Presence of a parent-specific allele in homozygous condition in any line is indicated by box shaded black and its absence by empty boxes. For example, the line DL1266-1 is homozygous for Vaishali specific allele of the marker Xgwm357-1A (column 2) and for SFW specific allele of marker Xgwm636-2A (column 3). Therefore, the boxes under 'V' and 'S' have been shaded black for columns 2 and 3 respectively. Each row of shaded boxes corresponding to one of the seven wheat lines thus represents their DNA fingerprints, which can be used as bar codes.

determine the proportion of parental genomic contribution to different progeny lines and to establish the correspondence between marker allele based selection and transmission of parental traits.

The Jaccard's similarity coefficient based on all the 33 marker loci ranged from 0.53 to 0.96 with an average of 0.76. This level of similarity based on STMS markers is much higher than that reported earlier in wheat[12]. The high average similarity was due to inclusion of seven sister lines and their parents in the study as compared to genetically divergent genotypes in the previous study as described earlier. Maximum similarity (96%) was evident between the sister lines DL1266-2 and DL1266-10. The breeding line DL1396-11 having a different pedigree was most divergent from others with an average similarity of 62%. The new plant type wheat lines along with the parents, SFW and Vaishali formed a major cluster with an average similarity of 81% (Figure 2) and remained isolated from the other varieties/lines. This cluster was further divided into two sub-clusters, corresponding to each of the parental lines. The Vaishali sub-cluster included the sister lines DL1266-2, DL1266-10 and DL1266-16 along with Vaishali. The SFW sub-cluster consisted of SFW and its daughter lines DL1266-5, DL1266-1, DL1266-17 and DL1266-6. The grouping of the new plant type wheat lines and varieties thus corresponded well to their pedigree and reflected the robustness of the mapped STMS markers in establishing genetic relationship.

The present study thus revealed the utility of a limited number of polymorphic STMS markers in differentiation of closely related lines supporting the earlier reports in bread wheat[4] and in other plant species including chickpea[14] in which low level of DNA polymorphism is known. The allele profiles across the eight polymorphic loci, which characterized the new plant type wheat lines, were used to graphically represent their genotypic constitution (Figure 3). As proposed earlier[15], these can be utilized along with the other markers, which were non-polymorphic in this set of genotypes, but in combination differentiated them from the other released varieties, as bar-coded molecular tags for the identification of the valuable new plant type lines as and when they enter either the breeding programs as donors, seed production chain as released varieties or the commercial market as consumer products. These markers would also be useful in quality testing and maintenance of purity of the seed samples.

1. Singh, S. S., Sharma, J. B., Chand, N. and Sharma, D. N., *Wheat Inf. Serv.*, 2001, **93**, 22–26.
2. Singh, S. S. and Singh, B. B., *IARI Wheats for Evergreen Revolution*, Indian Agricultural Research Institute, New Delhi, India, 2001.
3. Weber, J. K. and May, P. E., *Am. J. Hum. Genet.*, 1989, **44**, 388–397.
4. Plaschke, J., Ganal, M. W. and Roder, M. S., *Theor. Appl. Genet.*, 1995, **91**, 1001–1007.
5. Akagi, H., Yokozeki, Y., Inagaki, A. and Fujimura, T., *Theor. Appl. Genet.*, 1997, **94**, 61–67.
6. Struss, D. and Plieske, J., *Theor. Appl. Genet.*, 1998, **97**, 308–315.
7. Roder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.-H., Leroy, P. and Ganal, M. W., *Genetics*, 1998, **149**, 2007–2023.

8. Singh, S. S. *et al.*, *Ann. Wheat Newsl.*, 2002, **48**, 81–86.
9. Mohapatra, T., Sharma, R. P. and Chopra, V. L., *Curr. Sci.*, 1992, **62**, 482–484.
10. Rohlf, F. J., *NTSYS-PC. Version 2.0*, Exeter Software, Setauket, New York, 1998.
11. Law, J. R., Donini, P., Koebner, R. M. D., Reeves, J. C. and Cooke, R. J., *Euphytica*, 1998, **102**, 335–342.
12. Prasad, M., Varshney, R. K., Roy, J. K., Balyan, H. S. and Gupta, P. K., *Theor. Appl. Genet.*, 2000, **100**, 584–592.
13. Law, J. R., Cooke, R. J., Reeves, J. C., Donini, P. and Smith, J. S. C., *Plant Var. Seeds*, 1999, **12**, 181–190.
14. Udupa, S. M., Robertson, L. D., Weigand, F., Baum, M. and Kahl, G., *Mol. Gen. Genet.*, **261**, 354–363.
15. Ray Choudhury, P., Kohli, S., Srinivasan, K., Mohapatra, T. and Sharma, R. P., *Euphytica*, 2001, **118**, 243–251.

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A simple method for mass production of potato microtubers using a bioreactor system

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A simple protocol for mass propagation of potato microtubers was developed using an automated low-cost bioreactor system. Microtubers of potato were induced by a two-step culture method. In the first step (step A), the stock plants were inoculated in the bioreactor for growth and multiplication of plantlets. After four weeks, the medium was replaced with a new one to proceed to step B for microtuber induction. Comparative studies between solid and bioreactor culture (continuous immersion [with net or without net] and temporary immersion in liquid medium using ebb and flood) revealed that shoot multiplication and growth were more efficient in continuous immersion (with net) bioreactor. We also studied the effect of inoculation density on potato micropropagation during bioreactor culture and maximum responses were recorded when there were 50 nodal explants per bioreactor. After shoot proliferation, the culture medium was replaced with one containing a higher concentration of sucrose, with or without 6-benzylaminopurine (BAP) and kept under dark conditions. The analysis of tuber classification according to size showed that addition of BAP in the culture medium influenced the formation of microtubers larger than 1.1 g. It has also been observed that there is a strong influence of medium renewal on individual microtuber growth during bioreactor culture of potato. The results indicate that our system could be applied for mass propagation of potato tubers at low cost.

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AUTOMATION of organogenesis in a bioreactor has been advanced as a possible way of reducing cost of micropropagation[1–4]. Organogenic plant progagules are intensively cultivated in bioreactors to produce transplants for mass production. Intensive cultivation of potato microtubers and bulblets of lily is another strategy for producing progagules, which can be handled for direct planting in the field, thus facilitating commercialization. The main problems associated with microtuber production in conventional containers are the low yield of tubers and small tuber size that limits direct transplanting to field conditions. Recently, the adaptation of air-lift, bubble column, ebb and flow-type bioreactor (EFBR), and temporary immersion bioreactors for propagation of shoots and bud-clusters has provided a workable means for improving tuber quality and the number of tubers per plant[5–7]. Potato microtubers were produced in jar fermentors by semi-continuous liquid medium surface-level control[5]. Hulscher *et al.*[7] reported that 1600–1700 potato tubers can be produced by using an EFBR system, with a 10 l culture vessel in 18 weeks. Such systems are expensive, thus increasing the cost per propagule unit. One of the approaches to solve these problems is to simplify the culture system.

The aim of the present study was to establish an automated low-cost production system for microtubers and to investigate the different bioreactor systems (temporary immersion system using ebb and flood, continuous immersion system without net or with net), inoculation density, growth regulators and number of medium exchanges on the quality of the plants, and number and size of the tubers during potato shoot multiplication and tuber induction stages.

The meristem culture of potato 'Atlantic' was maintained onto MS solid medium[8] (3% sucrose + 2.4 g l^{-1} gelrite without phytohormone) and kept at 25°C, 70% relative humidity and a 50 µmol m^{-2} s^{-1} PPF (16 h/day). After four weeks of culture, nodal explants with one leaf were used for the experiments.

Potato microtubers were induced by the two-step culture method. In step A, the stock plants were inoculated in the bioreactor for growth and multiplication of plantlets. After three weeks, the medium was replaced with a new one to proceed to step B for microtuber induction.

Solid culture: Nodal explants (6 nodes per culture vessel) were inoculated to cylindrical culture vessels (0.5 l capacity) containing 150 ml MS medium containing 3% sucrose and 0.24% gelrite. The pH of the medium was