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Biotechnological Applications of Fertile, Transgenic Oat Plant

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Abstract

The resistance of Oat (Avena sativa L.) is low due to drought and salinity in the world's temperate cereal crop. A genotype-independent and efficient regeneration system using four oat cultivars, Prairie, Porter, Ogle and Pacer, was developed for genetically transforming the commercial oat cultivar available in this field. All these oat varieties produced multiple shoots from apical shoot meristems at a high frequency in vitro-independent genotype. This method was used to co-genetically transform three oat cultivars using biolistic TM bombardment with pBY520 (hva1 and bar) and pAct1-D (gus-containing). Herbicide tolerance and GUS markers have been used to identify and regenerate transgenic plants. The co-integration of hva1 and bar genes, at 100% and 61.6 percent of transgenic plants carried all three genes (hva1, bar and gus), is supported by molecular and biochemical analysis of putative transgenic plants. Further analysis of progeny R0, R1 and R2 verified that all of the transgenes are stable integration, expression and Mendelian heritage. A high GUS level of expression in vascular tissues and in the pollen grain of mature flowers has been shown in histo-chemical analysis of GUS protein in transgenic plants. In all stages of development, the immunochemical analysis of transgenic plants showed that hva1 was constitutive expression. In the early planting stages, however, HVA1 was higher.

Key words: Apical shoot meristems, Biolistic TM bombardment, GUS markers, Herbicide tolerance, Histo-chemical analysis, In vitro-independent genotype, Multiple shoots, Mendelian heritage, Oat (Avena sativa L.), Putative transgenic plants

Introduction

Oat is the seventh largest cereal crop in the global cereal production after wheat, maize, rice, barley, sorghum and millet, and ranks seventh in the year. Unlike other cereals, most oats are eaten at home and there is little trade in exports. Under cool and humid climates, oats flourish, but adapt to different soil types. The pH-adaptability of some varieties is higher than wheat and celiacs, from 5.5 to 7.0, but is even smaller as 4.5. The criteria for Oat are also small in lime requirement. But enough water is needed for growth and production of grain. Therefore, the major oat-producing countries are Russia, Canada, the United States, Finland and Poland. The Netherlands, Switzerland, West Germany, the UK, Ireland, Sweden, and France have the highest exchange rates for oats.

Oats have been an outstanding feed of livestock for centuries due to their high levels of protein and essential minerals. The high level of oats fiber also helps digestion. Oatmeal, oat starch and cookies are also used for human food [1]. The recent popularity of oats and oat bran as a health food is due to its features to minimize blood cholesterol and to the control of

gastrointestinal function. However, most recently, avenanthramides and beta-glucan are also in used in the cosmetics as a result of their two active compounds. Such compounds have been proved useful for the skin treatment and are used for the treatment of sunburn damage and as a method for skin regeneration. Oats are also used in paper and brewing, plastics, pesticides and preservative processing. Oat is also used as a cover crop or as hay, pasture, green manure. It improves soil life, eliminates weeds, controls erosion, and increases the organic components as a covering crop. All of this has led to greater demand for high-quality oats.

Climate pressures including drought and salinity are reducing worldwide farming productivity. In total, salinity affects about one-third of the world's irrigated agricultural land. Because of poor agricultural practices, Arable land is declining every day. For farmers, this is nothing but to grow in areas that are exposed to salinity and to harvest salinity-stressed plants. Oat is vulnerable to hot, dry weather and oat grain manufacture depends on adequate water availability in the growing season. There is something understood about the adaptability of oat to salinity. It is considered, however, relatively less tolerant to salt than other cereals or drilled plants. Several studies identify the reduced seed germination and subsequent development of various oat growers due to salinity. Nevertheless, the resistance of cereals or drillings is considered relatively lower than that of others. Hospitality is another obstacle to the production of oats. Oat may also be resistant to barley and insect pests in yellow dwarf viruses. Oat varieties were modified to produce pathogens resistance, but only at a small scale.

The maintained genomic history and inadequate selection methods of oats cannot solve this problem by traditional breeding. Nevertheless, in combination with the traditional breeding activities, new crop enhancement techniques, such as biotechnology, can be used to improve productivity. In addition to solve the existing problems, restricting crop production in developing countries, biotechnology provides exciting ways to promote sustainable agriculture. A smart selection of genetically engineered plants and the growth of resistant cultivars can therefore help increase oat yield in conditions of stress under genetic engineering.

Recent improvements in cereal cultivation depend on cultivating tissue and genetically modifying valuable trait genes. The knowledge on the in-vitro regeneration genotype independence and the oat genetic engineering method is limited. According to a researcher, who has developed an effective geno-independent regeneration method for the genetic transformation of commercial oat cultivars using apical shooting meristems. Subsequent studies describe use of Biolistc TM mediated oat-transformation system with mature, embryo-derived callous, young seedlings leaf base segments and embryogenic calli from immature embryos. The possibility of somaclonal variations in callus cultures due to prolonged tissue culture in embryo derived callus is, however, not considered viable in routine oat transformation. As an alternative genotype-independent regenerable objective tissue for genetic processing of the commercial oat cultivars, multiple shoot meristem crop

derived from mature seeds were therefore taken. The benefit of using this method means achieving the highest regeneration and reproducibility levels of target tissue. The maximum fertility rates and genomic stability in regenerated transgenic plants are also guaranteed. Here, it has been described the way apical meristems are manufactured and three oat growers transformed in a biological fashion, and their use to enhance osmotic stress tolerance using an osmotic stress-resistant hva1 gene. In addition, it has been identified that the transgenic oat plant molecular and biochemical analysis in order to confirm the transgenic integration, expression and inheritance stability, as well as the tolerance of salt and water deficit in transgender plant expressing hva1.

1. Requirement of Transgenic Plants:

Scientists are currently very excited about the possibilities of our growth in transgenic plant production and they are more looking forward to the day when this process is common, even in the case of oat. Nevertheless, it is important to first recognize and understand the unique genetic makeup of modern varieties of oat, irrespective of their country of origin, while trying to assess their impacts on oat development. Such varieties do not contain flaws in one or more features; they represent the best set of useful genes for the optimal use of an oat plant in its climate. The majority of new varieties are graduates of a rigorous series of field and laboratory research carried out over a number of years in many testing sites. They must have the proper nuclear gene combinations with a compatible and supportive cytoplasm to become commercially useful. It is rare and the active gene frequencies and variations in one population in different environments naturally will be different. When a breeder seeks to remedy a variety deficiency by creating hybrids between the oat breeder and a donor parent, the abundance of beneficial genes dilutes naturally because the donor plant produces half the genes. Although, donor plants are yet another commercial variety, a progeny with the production capacity of the best parental variety is often difficult to choose from such a hybrid. The function becomes enormous when the Donor parent and a significant wildlife is used to transfer genes to a hexaploid cultivary from a wild diploid or a tetraploid. Therefore, cross-cutting programs are so popular with breeders facing the problem of using such donor parents. This is also why oat breeders are so interested in genetically modified plants. Transformation technology aims at isolating and moving single important genes from almost any source into somatic or sexual cells in order to change the oat genome. The inclusion of a single gene in the unique combination of host genes, particularly in polyploids, is relatively less destructive. In the genome where it is expressed at the right time without creating a difference in overall gene expressions or in programming, the newly introduced gene must be integrated. Nevertheless, there is a possibility that one or more of those copies will interfere with the expression of other genes, thus harmful to the unique nature of the species if too many genes are introduced into the genome on different chromosomes. The plants that emerge from such a transformation could be quite complex, although a single gene is incorporated in transformed cell types, because the transgenes will presumably reside in any chromosome. In this situation, the breeder must rely on the traditional evaluation and selection procedures to obtain the desired transgenic phenotype. Useful genes of interest have

to be identified by screening gene libraries with different samples before sufficient transgenic experiments can be carried out.

2. Genetic probes:

Nowadays, in any geographical region, new varieties of oat include the combination of useful genes from adapted but deficit varieties with genes of one or more donor parents. Finally, phenotypically, either analyzes conducted by the breeder or biological tests are usually described. Molecular techniques allow breeders to check the donor genomes directly with RFLP, RAPD, or cloned gene samples for superior genes. The genomes are immediately improved by the breeders [2]. For example, disease resistance breeding generally requires progeny from a susceptible x resistant hybrid to be exposed to a pathogen in order to identify the plants that carry the resistance gene. Although this technique is usually effective, inoculations sometimes do not develop due to poor weather or inoculum conditions. The process takes time and energy and requires the completion of an entire life cycle for illnesses like smuts. Resistance may be found in the seedling process in other diseases such as rust. In either case, the combination or pyramid of two or more genes that are resistant to the same prevalent race is one of the breeding strategies employed. The other resistance gene prevents the spread of the new form if the fungus is able to overcome one of the host resistance genes by mutation or recombination. Although it is a good reproductive strategy, rust races that recognize resistance genes in the presence of other resistance genes which are often very difficult or nearly impossible to find. This is where the breeder will derive great value from genetic tests, since each gene or fragment of DNA will be detected by different tests closely related to each gene. In the case that the resistance genes required for all main races can be isolated and characterized, the breeder is able to select the best equipped resistance plants without using the fungal organism in the separating population. Nevertheless, in order to be effective, breeders must choose the most effective methods for selecting the desired progeny. The use of genetic samples can be costly, time-consuming and most cost-effective, if few species, such as a crossover system, need to be tested. When a large number of hybrid groups need to be tested for one or two races, the use of correctly selected races may be more effective instead of using genetic probes.

3. Tissue culture and Somaclonal Variation:

The success of oat farmers depends largely on genetic variation development by means of hybridization or mutation and the selection of useful and stable new genes [3]. The breeding group is involved in any process or strategy leading to new genetic variation. Variation may typically be observed and used by meiotic or sexual processes because they have a potential for reproduction. One fundamental principle of reproductive biology is the creation of a unitary or similar progeny through asexual reproduction because the process is based on mitotic divisions. This is the expected result, regardless of whether asexual reproduction occurs as part of an organism's normal life cycle, such as development of uredospores in rusts (Puccinia species), or man-made cloning of apical meristems, tubers, rhizomes or tillers [4]. Any irreversible genetic modification that occurs from such a procedure is rare and can

typically be identified or attributed through division or gene mutations for irregular chromosomal re-assortment. Interestingly, in well-defined nutrient media and in the regeneration of new plants of the callus cell or single tissue protoplast cells, physiologists and experts of tissue culture managed to develop undifferentiated callus tissue for many different plant organisms. Therefore, thousands of new plants from a single callus cell can now literally be produced. It also offers the possibility to grow anther-tissue haploid Calli and to supply tissue or cells with possible DNA transformation experiments [5].

4. Transformation:

i) Experimental transgenesis – early successes:

The development of technology for transgene transfer systematically from one species or variety to another without using normal sexual procedure is one of the priorities of modern biotechnology [6]. An individual plant receiving such a transgene is called as a transgenic plant, thereby incorporating it into its genome, displaying it through the nature of adequately RNA, protein or enzyme activity, and transmitting it to the progeny in normal sexual processes. The facility for the processing of transgenic plants varies widely with plant species. Some of these tasks are relatively simple, such as tobacco (*Nicotiana tabacum*). Dicotyledonous species including "potato (*Solanum tuberosum*), tomato (*Lycopersicum esculentum*), rapeseed (*Brassica spp.*), arabidopsis (*Arabidopsis thalliana*) and petunia (*Petunia hybrida*) were successfully used in the production of transgenic plants [7][8].

ii) Easy Transformation:

A number of steps have to be taken in order to synthesize transgenic plants. With one species which may or may not be effective procedures with other species cannot work. The first step is to classify and isolate the gene for use in a donor organism. It could be a gene that is previously isolated and used in the other organisms or one which has been altered by modifying its nucleotide sequence or nucleotide composition in the laboratory [9]. Molecular biology kits are now readily used to create a specific DNA plasmid building. The transgene consists in a linear order of three key parts: a promoter region, a significance gene coding sequence region and a terminator region. In order to promote the isolation of transformed cells or derivative tissues, the breeders may also want to attach a selectable marker gene such as antibiotic resistance or herbicide to recognize and confirm that the complete build has been successfully transferred (Fig. 1) [10]. The completed structure is now either directly or using a bacterial vector to be transferred into the host cells.

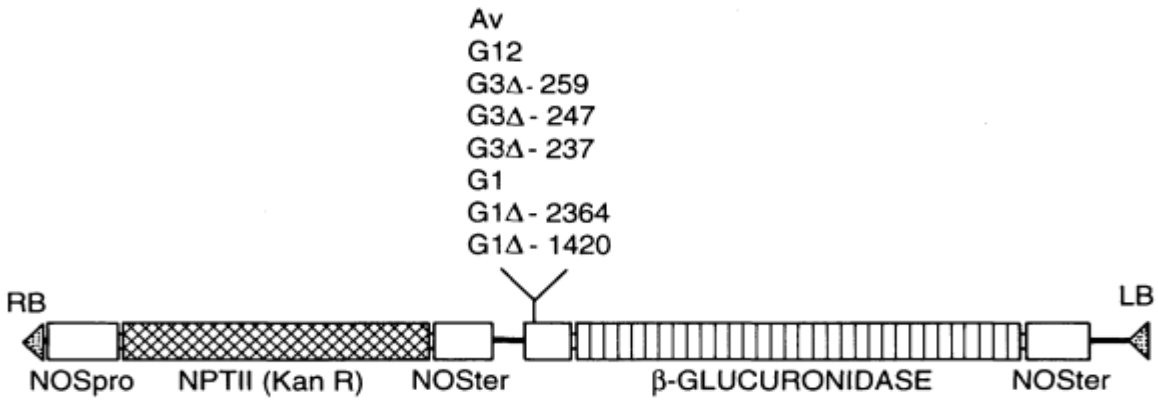

Fig.1: A typical DNA construct consisting of two transgenes used to study the biological activity of various oat storage protein gene promoter sequences in transgenic tobacco.

Conclusion

Oat science has already been affected by biotechnology. Some of the research studies has been carried out on other crops which has led to the basic knowledge and a summary of the promising methods of the work on oats. A transformation system has been established to allow the insertion into oats of foreign genes. An herbicide resistance gene was used to select transgenic oat plants. It is probably well-known that oat resistance to certain fungal toxins may allow the cloning and transformation of fungal toxin resistance genes. For example, the victorin resistance gene could serve as a useful selectable natural marker. DNA samples and hybridization techniques have been used extensively for the establishment of a genetic or molecular map of the oat genome in recent years.

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