TOWARDS MAPPING THE *DIOSCOREA* GENOME

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Abstract

Yams are important starchy tuber crops in (sub-)tropical countries of the world. Despite their importance in the regional economy, no serious attempt has been made toward their improvement. In order to obtain basic knowledge of the genetics of yams, we are trying to establish a linkage map of a wild yam species, *Dioscorea tokoro*. So far, six allozyme markers, six STMS markers and twenty AFLP markers have been identified. They will be used for linkage mapping of a population comprising 80 progeny obtained from a controlled cross.

1. INTRODUCTION

World yam production is about one-tenth of the total production of cassava and sweet potato [1]. Most of the yam crops are cultivated and consumed locally by subsistence farmers of tropical and subtropical regions around the world. Therefore, improvement of yam is important for enhancing sustainable agriculture in those regions where yams are cultivated. Despite its economical importance, so far no yam improvement has been carried out, apart from the selection of cultivars/clones currently under cultivation [2]. Breeding trials involving crossing experiments are not common [3,4], partly because of the dioecious nature, and partly because of the difficulty in crossing small flowers, of *Dioscorea*. Little information is available on the genetics of yams [4,5]. To improve our knowledge on yam genetics, we are presently constructing a linkage map of yam using a wild species, *Dioscorea tokoro* Makino.

2. MATERIALS AND METHODS

A wild yam species, *Dioscorea tokoro* Makino, belonging to the section *Stenophora* was chosen for linkage analysis. This species is widely distributed in East Asia, and has been used for population genetic studies [6]. It is a dioecious, diploid ($2n = 2x = 20$) species. Controlled crossing of *D. tokoro* is relatively easy. Under suitable conditions, a plant can grow from a seed to a mature plant within a year. These favorable features made *D. tokoro* an attractive model species for genetic studies on yams.

In 1995, we crossed, by hand pollination, a male (DT7) and female individual (DT5), both collected from the wild. This crossing gave rise to 200 seeds. Among them, 80 individuals were grown, and their sex, a single easily-scored morphological character, was determined when the flowers appeared. The leaves of the progeny were collected and used as source material for further genotyping. Parents used for the cross, as well as all the progeny, have been maintained through the years by vegetative propagation.

For linkage mapping, we have already developed six allozyme markers [6] and six STMS markers [7]. Variation in these genetic markers in the natural population of *D. tokoro* was studied. Additionally, we have started applying AFLP [8] markers, considering the high throughput of the method. Analysis of the segregation will be carried out by the pseudo-test cross strategy [9].

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3. RESULTS

Six allozyme markers (*Est, Got-1, Got-2, 6pgdh, Pgi* and *Skdh*) showed high genetic diversities (mean genetic diversity [10] was 0.38) [6]. Their segregation followed a simple diploid Mendelian fashion, although a segregation distortion was found in a locus *Pgi*. As expected, the STMS markers showed far larger genetic diversities (mean genetic diversity: 0.68) than the allozyme markers [7]. The higher genetic diversities imply a higher probability of the heterozygous state in *D. tokoro* genome. This high heterozygosity makes the application of the pseudo-test cross scheme [9] relevant for linkage mapping. These two subsets of markers are co-dominant markers, and should be useful for joining different family maps obtained with dominant markers, such as AFLP.

To further increase the number of useful genetic markers, we have started applying the AFLP [8] technique for yam genome mapping. First we have screened different primer combinations to see the levels of polymorphism detectable between the two parents used for crossing. Two commercially available kits for AFLP (AFLP analysis system I and II, recommended for organisms with genome sizes of 500 to 6000 Mbp/1C and of 100 to 500 Mbp/1C, respectively, Gibco BRL) were tested. These two systems are differentiated by whether the PCR primers have a 6-base selective anchor (system I), or 5-base selective anchor (system II) at their 3' ends. After applying 8 primer combinations for each kit, we have detected a total of 203 (52 polymorphic) fragments for kit I, and total of 432 fragments (81 polymorphic) fragments for kit II. Optimal results were obtained by kit II. This may reflect the relatively small genome size of yam species (555 Mbp/1C reported for *D. alata* [11]). The number of polymorphisms detected between the two parents is satisfactory, considering the possible number of primer combinations available.

Using two selected primer combinations (*Eco*RI-AC x *Mse*I-CAC and *Eco*RI-AA x *Mse*I-CTC), we studied the segregation of AFLP among progeny obtained by the controlled cross. A total of 26 polymorphic fragments were detected between the two parents (Table I). They could be grouped into five different categories in terms of presence or absence of the fragments in either parent and the segregation among progeny. Only the polymorphisms grouped in the categories 1 and 2 are useful for linkage mapping with the pseudo-test cross scheme [9]. Thus, 18 AFLP fragments remain for further linkage analysis. Considering the fact that two linkage maps are constructed, each corresponding to one parent, we should divide this number by two to represent the number of useful polymorphic fragments per linkage map. Therefore, we have 4.5 useful AFLP fragments for mapping per primer combination per parent, if the results obtained for the two experiments can be regarded as representative. This means only 25 AFLP experiments are needed to obtain 100 useful markers for linkage mapping, confirming a high throughput of the AFLP technique, and its applicability to obligately outcrossing species such as yams.

4. DISCUSSION

The co-linear arrangement of genomes (so-called synteny) has been reported in Gramineae and Fabaceae plants. There is a high probability that this is also the case among *Dioscorea* species. Therefore, the linkage map obtained for *D. tokoro* in this current study will be helpful to make linkage maps in other economically important yam species. To further facilitate the transfer of linkage information from *D. tokoro* to other species, selected polymorphic AFLP fragments would have to be cloned and sequenced. Development of

various new genetic markers have now made it possible to embark on the molecular-marker-based breeding in yams, a long neglected crop.

TABLE I. OCCURRENCES OF FIVE CATEGORIES OF AFLP'S OBSERVED AMONG PARENTS AND PROGENY OF *DIOSCOREA TOKORO*

Category	Female parent (DT5)	Male parent (DT7)	Segregation among progeny	No. of occurrences
1	+	-	Yes	10
2	-	+	Yes	8
3	+	+	Yes	5
4	+	-	No	2
5	-	+	No	1
Total No. of polymorphic fragments				26

(+: fragment present, -: fragment absent) ; The results for the two AFLP reactions (*Eco*RI-AC x *Mse*I-CAC and *Eco*RI-AA x *Mse*I-CTC) are pooled.

ACKNOWLEDGEMENTS

This research was supported by fellowship No. IV-1-7121-1028559 to RT from the Alexander-von-Humboldt Foundation, Germany, for which we express our cordial gratitude.

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