

Received 18 February 2002  
Accepted 11 June 2002

Short Communication

**MOLECULAR MARKERS OF INBRED RADISH (*Raphanus sativus* var.  
*Radicola* Pers.) LINES**

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**Abstract:** An approach which could be used for quick searches for RAPD markers is described for groups of radish lines with certain morphological traits. The lines are characterized by various morpho-physiological abnormalities, including tumor formation (lines 12, 19, and 21) and non-terminal development of the flower meristem as a variant of tumor growth (line 6). We found four markers which differentiate tumor radish lines 12, 19, and 21 from the others, and two which differentiate line 6.

**Key Words:** *Raphanus sativus*, RAPD Markers, Genetic Collection

**INTRODUCTION**

Radish is an important crop and a useful model for developmental genetics. It is a diploid species with a relatively small genome. Besides this, it also exhibits a number of interesting abnormalities: dwarfism/gigantism, agravitropic growth, vivipary, wilting, crop-root cracking, non-terminal development of the flower meristem, and tumor formation on the crop-root [1]. These are all present in the genetic collection created in our department from single plants of Saxa and Virovsky Bely cultivars by selfing for more than 30 generations. Nevertheless, the amount of known markers is not great enough to enable the construction of a genetic map of the species. The list of markers for genetic mapping may be lengthened via the use of molecular markers. In addition, finding molecular markers tightly linked with a given morphological trait helps us to better understand that trait's molecular basis.

The aim of this research was to find markers for two groups of radish lines: lines exhibiting the formation of tumors on the crop root and a line with non-terminal development of the flower meristem. Modified bulked segregant analysis [2] was used, and is described below.

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## MATERIAL AND METHODS

In this study, we used radish lines derived from two cultivars - "Virovsky Bely" (Nos 5, 6, 10, and 12) and "Saxa" (Nos 18, 19, 21, and 30). Lines 10, 12, 19, and 21 have tumor formation on the crop root. Lines 5, 18, and 30 do not exhibit tumor formation. Line 6 has non-terminal development of the flower meristem and does not exhibit tumor formation.

Plant DNA was isolated according to a modified Scott and Draper procedure [3]. We used primers, produced by Operon (A8, A15, A20, B3, B5, B10, C6, C13, D13, E2, E13, F14, F20, G9, G12, H4, H12, I10, J7, J15, J16, K1, K6, K8, M1-M20, Q15, S18, T1-T20, V10, W13, X4, X20, Z4, Z7, Z18, Z20), and Roth (A1-A20), and primers with following sequences: A (ccgcatctac), B (gtccccgacga), C (cggaccggtg), D (gttgccagcc), E (accgcaagg), F (ggaccaacc), G (gtcgccgtca), H (agcgccattg), I (gagagcccac), J (agatgcagcc), and some 2-primer combinations. Each 50 $\mu$ l polymerase chain reaction contained 30 ng of isolated DNA, 2.5 U of Taq polymerase (Sileks M), a buffer supplied by the enzyme manufacturer, 20  $\mu$ M of each dNTP, and 10 pM of primer. PCR was carried out for 1 cycle of 5' at 93°C, 5' at 33°C, 5' at 72°C, 33 cycles of 1' at 93°C, 1' 30'' at 33°C, 2' at 72°C, followed by a final extension for 5' at 72°C. PCR fragments were separated on 1% agarose gel in the presence of ethidium bromide.

## RESULTS AND DISCUSSION

Four molecular markers were identified for radish tumor lines using a modification of BSA, described below. This approach contains three main steps and permits the performance of several times fewer PCR reactions than are needed when each line is analysed independently. In the 1<sup>st</sup> step, PCR was performed using a mixture of DNA from all the tumor lines (mixture 1) and from all the non-tumor lines (mixture 2). Products were obtained using 128 of the 142 primer and 2-primer combinations. 15 of them showed differences among (between) the reaction products. They were used in the 2<sup>nd</sup> step, where PCRs were done on the DNAs of each line separately. The aim of the experiment was to find RAPD- markers which are similar for all the tumor lines and different from that of all the non-tumor lines.

Such markers were found using primers J7, M9, T17, and B-T9, and were named the j7, m9, t17, and bt9 markers of lines 12, 19, and 21. In the 3<sup>rd</sup> step, they were genetically characterized. We investigated the co-inheritance of RAPD markers with exhibition of tumor formation, and of RAPD markers with each other in a hybrid combination 18x19. No linkage was found among these markers (Tab. 1). Similar analysis was done for non-terminal development of the flower meristem. We found 2 markers with A15 and G primers, which differentiate lines 6 (mutant) and the wild type. An analysis of the co-inheritance of these markers with morphological traits in hybrid combination 6x5 showed independent

inheritance of the non terminal development of the flower meristem with g ( $\chi^2_{9:3:3:1} = 0,45$ ) and a15 ( $\chi^2_{9:3:3:1} = 2,80$ ).

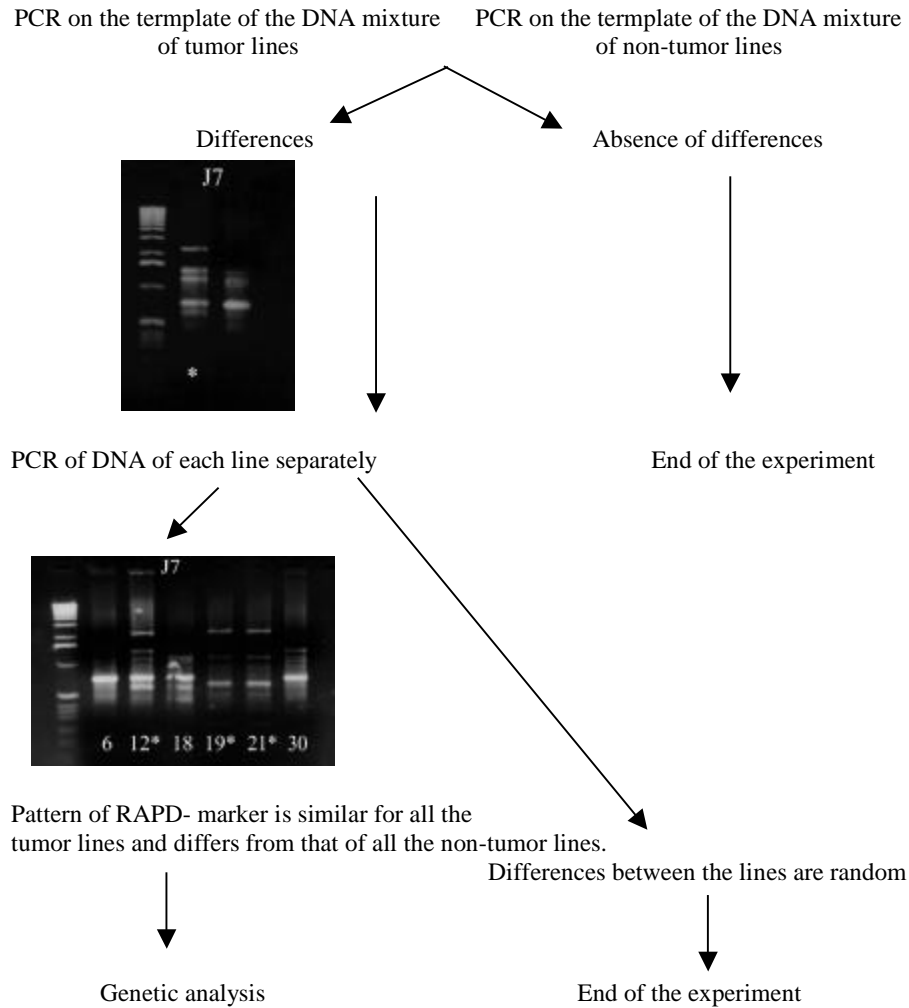


Fig. 1. Scheme of the RAPD analysis of *Raphanus sativus* lines. Results of the PCR of the tumor lines are marked by a \*. The 1<sup>st</sup> line on each gel is a 1 kb ladder, with the primers and numbers of the lines indicated on the photo.

In this study, we have shown the polymorphism of RAPD-markers for radish lines. For radish lines 12, 19, and 21, we found the markers j7, m9, t17 and bt9, and for line 6 with the non-terminal flower meristem, we found markers a15 and g.

Tab.1. Co-inheritance of analysed traits in 18x19 F<sub>2</sub> hybrids: values of  $\chi^2_{(9:3:3:1)}$  for pairs of markers

markers	tumor	j7	t17	m9
j7	3.44			
t17	5.53	0.38		
m9	7.31	6.34	3.56	
bt9	7.60	6.10	4.66	1.38

The data obtained will be the basis for constructing a genetic map of radish. When the list of RAPD-markers for each group of radish lines is enlarged, it will be possible to find links between some of them. The most interesting of the expected results is the finding of a tight link between morphology and the RAPD-marker, as it will clarify the nature of the morphological abnormality.

**Acknowledgements:** The research described in this publication was made possible in part by Award No. ST-012-0 of the U.S. Civilian Research and Developmental Foundation for the Independent States of the Former Soviet Union (CRDF).

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