Effect of the MAR chicken lysozyme A element on the expression of the endochitinase gene from *Trichoderma harzianum* in *Brassica napus*

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ABSTRACT

Matrix attachment regions (MARs) are defined as genomic DNA sequences, located at the physical boundaries of chromatin loops. They are suggested to play a role in the cis unfolding and folding of the chromatin fibre associated with the regulation of gene transcription. Studies on the expression of reporter genes in transgenic plants indicated that MARs can increase transgene expression levels and reduce the variability of transgene activity. Here we report about the influence of the MAR chicken lysozyme A element on the expression of the agronomic interesting gene endochitinase from *Trichoderma harzianum* in *Brassica napus*. We have produced by *agrobacterium* mediated transformation a population of 47 primary transformants containing the endochitinase gene and a second population of 48 primary transformants containing the endochitinase gene with MAR elements at the T-DNA borders. The endochitinase gene was under control of a doubled 35S promoter. The presence of the MAR element increased slightly the average endochitinase activity and the number of plants with a high transgene expression level within the population of primary transformants in comparison to the control without MARs. The effects of the MAR elements were more obvious in single copy plants

Key words: Matrix attachment regions, position effect, transgenic, endochitinase

INTRODUCTION

It has long been observed that the level of transgene expression in transformants can vary over a wide range. The variability is known as 'position effect', because it is supposed to be due to the random place of integration of the transgene in the plant genome. Matrix attachment regions (MARs) are suggested to play a role in the cis unfolding and folding of the chromatin fibre associated with the regulation of gene transcription. The influence of MAR elements on the expression of reporter genes were already shown in tobacco, rice, poplar, barley and maize. The studies indicated that MARs can increase transgene expression levels and reduce the variability of transgene activity. Not all MARs have the same mode of action. Observed effects are likely to depend on the MAR itself, the transgene, the assay system, transformation approach and the recipient cells. In this study we investigated the influence of the chicken lysozyme A element on the transgene expression in oilseed rape. It was shown in tobacco that this MAR element reduced the variation of the reporter gene activity significantly and increased the average activity (Mlynárová et al., 1994).

MATERIALS AND METHODS

5 different oilseed rape cultivars (Drakkar, SR163, Pactol, WRG115, Lizard) were transformed with *Agrobacterium* C58ATHV carrying the plasmid of choice (Fig.1) by the hypocotyl transformation procedure according to Damgaard et al. (1997) with minor modification. From the transformation with pBINESR 47 RC plants were regenerated. From the transformation with pBIN∆ESR 48 RMC plants were regenerated containing the chicken lysozyme A Element at the T-DNA borders. The activity of the endochitinase in *in vitro* plants were determined following the protocol described in Mora and Earle (2001). Total DNAs from transformed and untransformed greenhouse plants were isolated by the method of Rogers and Bendich (1988). Southern blot analysis was carried out according to McCabe et al. (1997), total DNAs being digested with *Eco*RI and hybridised with a DIG labelled endochitinase specific probe.

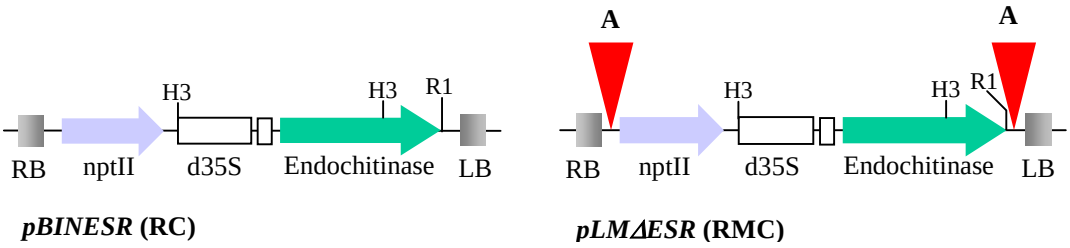

Fig. 1. Overview of the T-DNA region of vectors used in the experiments
d35S: double CaMV 35S promoter, A: MAR chicken lysozyme element A

RESULTS

To evaluate the influence of the MAR chicken lysozyme element A in oilseed rape we have produced by *agrobacterium*-mediated transformation a population of 47 primary transformants containing the endochitinase gene and a second population of 48 RMC plants containing the endochitinase gene with the MAR chicken lysozyme A element at the T-DNA borders. The average endochitinase activity of the RMC (MAR) plant population was 106,6 ± 78,7 pmol min^{-1} μg^{-1} and varied from 0 to 335 pmol min^{-1} μg^{-1}. The average endochitinase activity of the RC (no MAR) plant population was 80,5 ± 65,7 pmol min^{-1} μg^{-1} and varied from 0 to 284 pmol min^{-1} μg^{-1}. There was no significant difference in variation in transgene expression levels between independent transformants of the 2 analysed populations. The frequency distribution of endochitinase activity (Fig. 2) shows one maximum for the RC (no MAR) plants at class 0-50 U. Remarkably, the distribution of the RMC (MAR) plants showed a second maximum at class 150-200 U. The population of the RMC (MAR) transformants contained more plants with high transgene expression level in comparison to the RC (no MAR) population. 22 plants of each population were analysed by Southern Blot. In both groups 13 single copy events were detected, respectively. In Fig. 3 the endochitinase activity was plotted against the copy number. The effect of the MAR element was more obvious considering only the one copy plants. The MAR containing plants show a reduced variation and a increased average transgene expression level in comparison to the control plants.

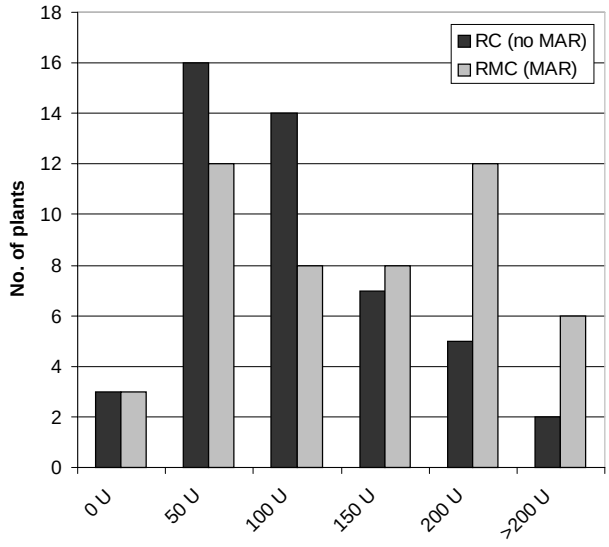

Fig. 2. Frequency distribution of endochitinase activity.
The number of plants is plotted against the endochitinase activity. Plants are grouped into classes, the highest activity of which given on the x-axis. The enzyme activities in the class labelled 100 contain all plants with an activity ranging from 51 to 100 pmol min^{-1} μg^{-1}.

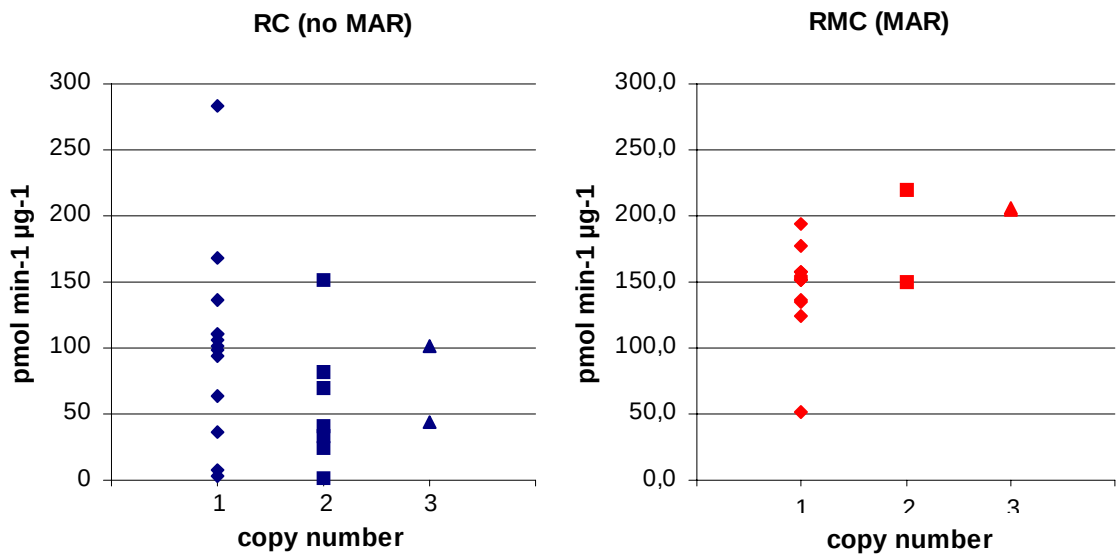

Fig. 3. Endochitinase activity as a function endochitinase gene copy number

DISCUSSION

In this work we have investigated the influence of the chicken lysozyme element on transgene expression in oilseed rape. Mlynárová et al. (1994) showed that the effect of this MAR were most pronounced when the elements were placed next to the borders of the T-DNA. By using this T-DNA design we could show a slight increase in average endochitinase activity and a higher proportion of plants with high enzyme activity within the MAR population. However, we could not find a significant difference in variation in transgene expression levels as reported in tobacco. It is known that the effect of a MAR element depends on several parameters e.g. promoter and recipient cell. Mlynárová et al. (2002) have shown that the MAR A element reveals a more obvious effect on reduction of variation in combination with the Lhca3 promoter than in combination with the 35S promoter which was used in this study. Single copy plants containing MAR A elements show a decrease in variation in endochitinase activity in comparison to single copy plants of the control population. This finding is consistent with the MAR chromatin hypothesis, which predicts that transgenes flanked by MAR sequences would form an independent loop domain and their expression would be insulated from the influence of the surrounding chromatin.

ACKNOWLEDGEMENTS

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