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A rice homeotic gene, *OSH1*, causes unusual phenotypes in transgenic tobacco

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A rice gene, *OSH1*, which shares homology with animal homeobox genes, has been isolated. We have introduced the *OSH1* cDNA into tobacco in order to examine its function. Expression of the *OSH1* cDNA in tobacco induced morphological abnormalities in the leaves, petals and stems of the transformants suggesting that *OSH1* functions as a morphological regulator. *OSH1* cDNA expression was analyzed under the control of three different promoters. This work revealed that not only the level of *OSH1* expression but also the site and timing of the expression affect the morphology of the plant.

Plant development; Morphological change; Transgenic tobacco; Homeobox

1. INTRODUCTION

The molecular mechanisms which determine the morphology of organs in living organisms has long been one of the fundamental questions of biology. In 1983 the first gene involved in eukaryotic development was isolated from *Drosophila* [1]. Since then, a large body of work has been conducted to reveal the molecular functions of these homeotic genes and how they form a multicellular organism composed of differentiated cells (reviewed in [2]).

Unlike animals, higher plants maintain groups of undifferentiated cells or meristems throughout the life cycle. The cells of the shoot apical meristem maintain themselves in an undifferentiated state and progressively give rise to leaves and floral organs. One question is whether or not homeotic genes control these development and differentiation processes in plants in the same way as in animals. There are many mutations known to affect plant morphology, but the molecular mechanisms responsible for these effects are poorly understood.

A dominant mutation which alters maize leaf development, knotted-1 (*KN-1*), was first identified many years ago [3]. Recently, the *KN-1* gene was isolated by transposon tagging and shown to encode a homeodomain protein which is conserved throughout the homeobox gene family [4]. This was the first gene reported in plants to encode a homeodomain protein. It is also the only homeobox gene known to be involved in the development of a vegetative meristem. This finding hints that homeobox genes may control plant development as in animals. Previously, we isolated a homeobox-containing gene, *OSH1*, from rice [5]. Here, we introduced it into tobacco under the control of three different promoters. The transformed plants show a range of phenotypes which include abnormalities in leaf and petal shape as well as stem height and number. The results we describe here suggest that *OSH1* functions as a regulator of morphological development in tobacco. Furthermore, the site and stage of *OSH1* gene expression seems to be important in determining plant morphology.

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2. MATERIALS AND METHODS

2.1. *Plant material and transformation procedure*

Transformation and regeneration of *Nicotiana tabacum* cv Samsun NN were performed as previously reported [6].

2.2. *Construction of chimeric genes*

A cDNA clone encoding rice *OSH1* was introduced into the *XbaI*/*SacI* site of pBI121 (Clontech Laboratories Inc., CA, USA) to construct 35S-*OSH1*. The promoter region was replaced with the nopaline synthase promoter (NOS) [7] or the promoter of the pathogenesis-related protein 1a gene (PR) [8] to make NOS-*OSH1* and PR-*OSH1* fusion genes, respectively. A control construct with the β-glucuronidase gene driven by the CaMV35S promoter (35S-GUS) was used as well.

2.3. *RNA blot hybridization*

RNA was extracted from mature leaves of each transformant with the method using guanidium thiocyanate [9]. Total RNA (10 μg from each transformant) was applied to a 1% agarose gel. Hybridization with the coding region of radio-labeled OSH1 was analyzed by autoradiography.

3. RESULTS

The expression of all three *OSH1* constructs introduced into tobacco resulted in abnormal morphology in

the transformed plants. This transformation method has previously been used to generate hundreds of transgenic tobacco plants, and none of those transformants have had morphology similar to that produced in the *OSH1* transformants. This indicates that the abnormal morphology observed in the *OSH1* transformants is due to the introduced gene, rather than to somaclonal variation.

As shown in Fig. 1, the morphology of the transgenic plants can be divided into three categories ranging from mild to severe in phenotype. Plants with the mild phenotype have wrinkled leaves (Fig. 1b) and the leaves are thicker and shorter in length and are more disc shaped compared to wild-type leaves (Fig. 1a). The plants with intermediate phenotypes have elongated stems and slender leaves (Fig. 1c) and the older leaves are wrinkled, similar to those found on plants with the mild phenotype. The plants with severe phenotypes are dwarf and the axillary buds develop into vegetative stems (Fig. 1d); these buds are dormant in wild-type plants. The leaves

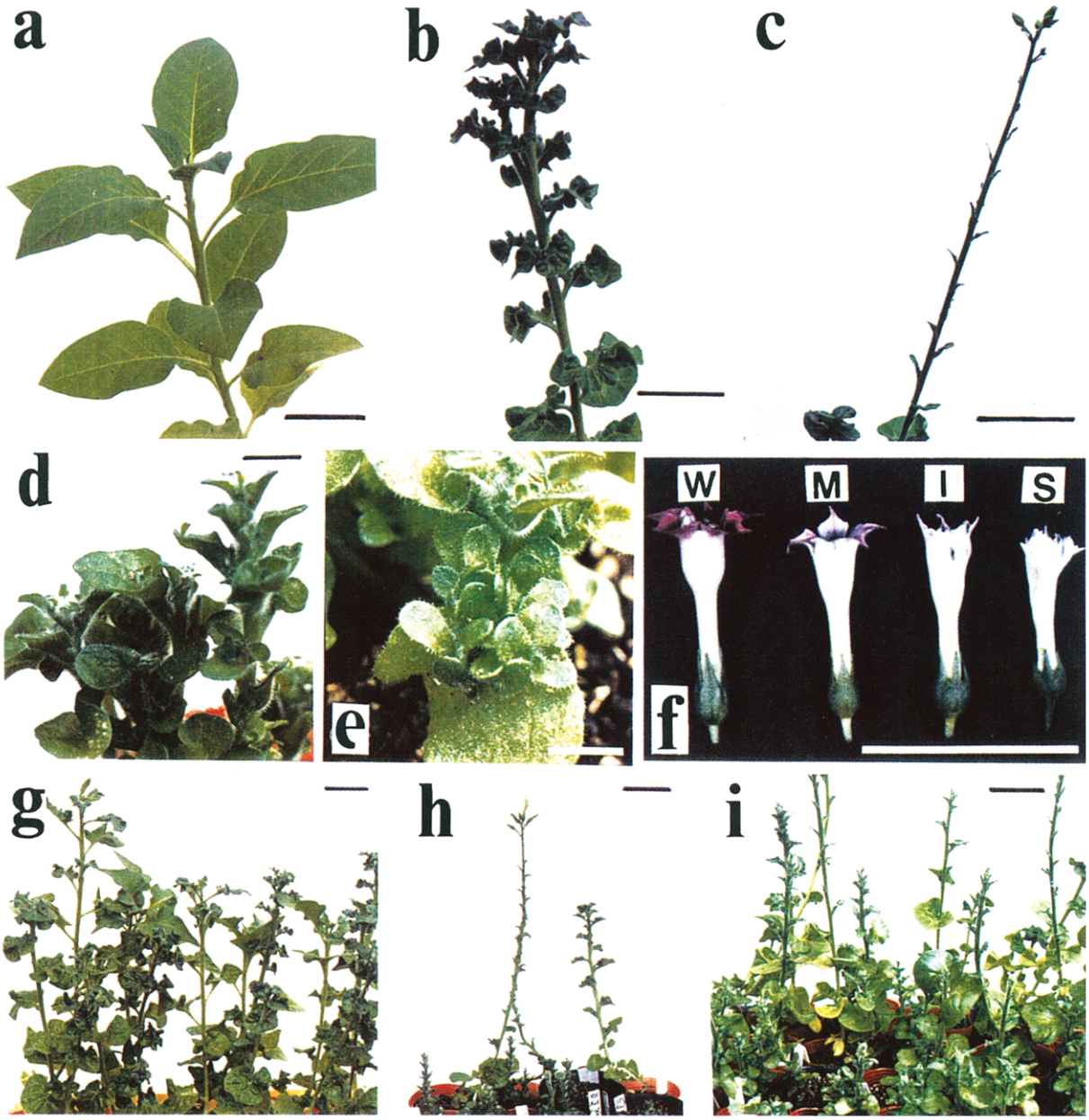

Fig. 1. Phenotypes of the transgenic plants. (a) Wild-type (untransformed) tobacco. (b) The mild phenotype with wrinkled leaves. (c) The intermediate phenotype has elongated stems and slender leaves. (d) The severe phenotype is dwarf and the axillary buds develop into leaves and/or shoot apices. (e) Developing shoot apices on the leaves of plants with the severe phenotype. (f) Flowers of transformants with mild (M), intermediate (I) and severe (S) phenotypes and wild-type (W). The NOS-*OSH1* transformants primarily have the mild phenotype (g). The 35S-*OSH1* transformants can be divided into two categories, mild and severe phenotypes (h). Approximately 80% of the PR-*OSH1* transformants have an intermediate or severe phenotype (i). Bar indicates 5 cm except in (d) and (e), which indicates 1 cm

on these plants are not wrinkled, but they are tiny and occasionally show abnormal shoots on the leaf (Fig. 1e). As tobacco has strong apical dominance, these events must be due to loss of apical dominance.

Morphological abnormalities in flowers have also been observed, which correlate with the aberrant leaf shapes (Fig. 1f). As the leaf phenotype increases in severity, the flower color becomes increasingly pale and the flower margin becomes dissected. Flowers on plants with the mild phenotype have only a bit of pink color at the tips of the flowers and the margins are slightly dissected. Flowers on plants with the intermediate phenotype are very pale and are more dissected than those of the mild phenotype. The tube is also slightly wrinkled. Flowers on plants with the severe phenotype are almost white and somewhat shorter in length than a wild-type flower. The margins are severely dissected, and the tube is compressed around the central structures. Although the stamens of flowers with the mild phenotype are almost normal, the anthers are shrunken in comparison to those of the wild-type flower. The intermediate type have shorter stamens with swollen anthers. Flowers on plants with the severe phenotype have short, curled stamens and shrunken anthers. The number of stamens and petals is normal in all of these transformants. Interestingly, although *OSH1* expression is responsible for many abnormalities in the stamens and petals, the stigma and calyx remain essentially normal.

Most of the NOS-*OSH1* transformants express the mild phenotype (Table I and Fig. 1g). Only one of 27 transformants examined expressed the intermediate phenotype and none expressed the severe phenotype. In contrast, 35S-*OSH1* plants result in two phenotypes. Almost half of the transformants express the severe phenotype and the other half are either normal or only mildly affected (Table I and Fig. 1h). About one-third of the PR-*OSH1* transformants express the severe phenotype while half are of the intermediate phenotype. Less than 20% of the transformants show the mild phenotype (Fig. 1i) and only 1 normal plant was observed among 36 transformants (Table I). The difference in the severity of the phenotype observed with different promoters might be attributable to *OSH1* expression level. Therefore, we analyzed *OSH1* expression by RNA blot hybridization.

Table I

Phenotypic categories of transformants

	Phenotype			
Constructs	Normal	Mild	Intermediate	Severe
NOS-*OSH1*	5 (18.5%)	21 (77.8%)	1 (3.7%)	0 (0.0%)
35S-*OSH1*	6 (26.1%)	6 (26.1%)	1 (4.3%)	10 (43.5%)
PR-*OSH1*	1 (2.8%)	7 (19.4%)	17 (47.2%)	11 (30.6%)

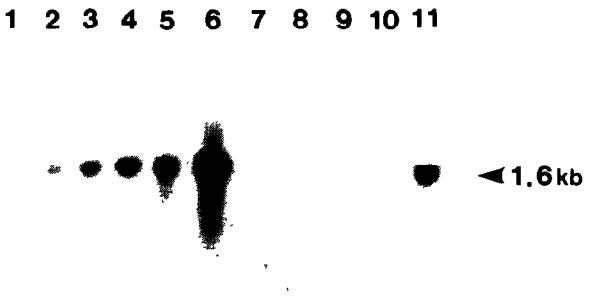

Fig. 2. RNA blot hybridization. Ten μg of total RNA from mature leaves of each transformant were applied to a 1% agarose gel. (Lane 1) Wild-type. Lanes 2–4 are from plants transformed with NOS-*OSH1*. (Lane 2) Mild phenotype but leaves only slightly wrinkled. (Lane 3) Mild phenotype with typically wrinkled leaves. (Lane 4) Mild phenotype with extremely wrinkled leaves. Lanes 5 and 6 are from plants transformed with 35S-*OSH1*. (Lane 5) Mild phenotype with typically wrinkled leaves. (Lane 6) Severe phenotype with many shoots. Lanes 7–11 are from plants transformed with PR-*OSH1*. (Lane 7) Mild phenotype with slightly wrinkled leaves. (Lane 8) Mild phenotype also with typically wrinkled leaves. (Lane 9) Mild phenotype with slender leaves. (Lane 10) Mild phenotype with severely wrinkled leaves. (Lane 11) Severe phenotype with many shoots.

RNA blot analysis was undertaken with RNAs extracted from mature leaves of each phenotype to determine the expression level of *OSH1*. In all of the transformants analyzed there was only one transcript of 1.6 kb in size corresponded to the *OSH1* mRNA. No signal was detected in RNA extracted from untransformed tobacco (Fig. 2, lane 1). In plants transformed with NOS-*OSH1* and 35S-*OSH1*, those with the severe phenotype show increased levels of *OSH1* expression in abnormal leaves (Fig. 2). In plants with the mild phenotype, the expression level of *OSH1* increases as the wrinkle in the leaves becomes more severe (lanes 2–5). In the 35S-*OSH1* transformants *OSH1* expression is higher in plants with the severe phenotype (lane 6) than in plants with the mild phenotype (lane 5). In contrast, *OSH1* expression in the PR-*OSH1* transformants (Fig. 2, lanes 7–11) was observed only in the leaves of plants with the severe phenotype (lane 11). No expression was detected in the leaves of plants with the mild phenotypes. These results suggest that there is no simple correlation between the level of *OSH1* gene expression in the mature leaf and the severity of the morphological abnormalities in leaf shape.

4. DISCUSSION

Introduction of the rice *OSH1* gene into tobacco clearly affects the morphology of tobacco shoots, leaves and flowers, thus it appears that the *OSH1* gene product functions as a morphological regulator in tobacco. Very recently, similar results were obtained with maize *KN1* gene controlled under 35S-promoter in transformed to-

bacco [10]. The levels of *OSH1* expression and the severity of morphological abnormality show interesting correlations. *OSH1* expression from the NOS promoter parallels the degree of morphological aberration in that transformants with more severe phenotypes have higher levels of *OSH1* expression. This is also true for *OSH1* expression from the 35S-promoter, which is known to be expressed throughout the plant, although the strongest expression is found in roots [11]. Plants with the severe phenotype show dramatic expression of *OSH1* which is more than ten times than that observed in plants with the mild phenotype. Taken together, these results appear to indicate that the degree of morphological change depends on the level of *OSH1* expression. This conclusion, however, cannot be drawn from the results obtained with the PR-*OSH1* construct.

Tobacco plants transformed with PR-*OSH1*, whether the phenotype is mild or intermediate, contain extremely low levels of the *OSH1* transcript in the leaves. There is essentially no detectable expression of this transcript in mature leaves. This raises a question as to why do many of these transformants have such dramatic phenotypes. It is possible that *OSH1* expression is localized and is restricted to early stages of development. The PR1a promoter is known to be activated by viral infection or by salicylic acid-treatment [12–14]. It is also expressed in the early stages of leaf or flower development, but is completely inactivated in mature leaves (Ohashi et al., unpublished data). We have also found that the only leaves which show *OSH1* expression are found on plants with many shoot apices (Fig. 2, lane 11). Together, these results suggest that *OSH1* need not be expressed all the time or in the entire plant in order to result in morphological aberrations. It seems that expression of *OSH1* in the early stages of lamina development, i.e. expression in the shoot apical meristem, is sufficient for the induction of morphological abnormalities.

The localization of *OSH1* expression may determine the fate of the meristem and may be one of the first triggers of differentiation. Histochemical studies show that the KN1 protein is expressed in the nuclei of an apical meristem as long as it remains undifferentiated but disappears when the meristem shows signs of differentiation [15]. *KN1* and other homeotic genes are likely to affect development as *trans*-acting factors [16], therefore, *OSH1* may regulate tobacco development in a similar manner. The identification of a target gene(s) for *OSH1* will help to elucidate the network of regulatory process controlling plant development.

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REFERENCES

[1] Gaber,R.L., Kuroiwa, A. and Gehring, W.J. (1983) EMBO J. 2, 2027– 2036.
[2] Gehring, W.J. (1987) Science 236, 1245–1252.
[3] Bryan, A.A. and Sass, J.E. (1941) J. Hered. 32, 343–346.
[4] Hake, S., Vollbrecht, E. and Freeling, M. (1989) EMBO J. 8, 15–22.
[5] Matsuoka, M., Ichikawa, H., Saito, A., Tada, Y., Fujimura, T. and Kano-Murakami, Y. (1993) Plant Cell 5, 1039–1048.
[6] Matsuoka, M. and Sanada, Y. (1991) Mol. Gen. Genet. 225, 411–419.
[7] Bevan, M., Barnes, W.M. and Chilton, M.-D. (1983) Nucleic Acids Res. 11, 369–385.
[8] Ohshima, M., Matsuoka, M., Yamamoto, N., Tanaka, Y., Kano-Murakami, Y., Ozeki, Y., Kato, A., Harada, N. and Ohashi, Y. (1987) FEBS Lett. 225, 243–246.
[9] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[10] Shinha, N., Williams, R. and Hake, S. (1993) Genes and Development 7, 787–795.
[11] Benfey, P.N., Ren, L. and Chua, N.-H. (1989) EMBO J. 8, 2195–2202.
[12] Hooft van Huijsduijnen, R.A.M., Cornelissen, B.J.C., Van Loon, L.C., Van Boom, J.H., Tromp, M. and Bol, J.F. (1985) EMBO J. 4, 2167–2171.
[13] Pftzner, U.M. and Goodman, H.M. (1987) Nucleic Acids Res. 15, 4449–4465.
[14] Matsuoka, M., Asou, S. and Ohashi, Y. (1988) Plant Cell Physiol. 29, 1185–1192.
[15] Smith, L., Greene, B., Veit, B. and Hake, S. (1992) Development 116, 21–30.
[16] Levine, M. and Hoey, T. (1988) Cell 55, 537–540.