Correlated Expression of *gfp* and *Bt cry1Ac* Gene Facilitates Quantification of Transgenic Hybridization between *Brassica*s

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Several mistakes appeared in the above-mentioned paper. The data were collected by different persons, i.e., one collected GFP fluorescence and another measured Bt protein and the developmental stages were incorrectly labelled. It was assumed that the data at various leaf stages were disordered and the actual order of the developmental stages should be the inverse to that described in the paper. As a consequence, the overall trend of GFP and Bt protein concentration *increased* along with the growth of plants rather than decreased, although their expression is still varied. The disorder of the data also affected the correlation formulae between GFP and Bt protein but did not affect their associated relationship. Thus the corrected data are provided as follows.

Materials and Methods

GFP quantification

For all hybrids, the top mature leaf was sampled at the two-leaf stage, and the third upper-most leaf was measured at the four-leaf and six-leaf growth stages to quantify GFP dynamics as plants aged.

Results

The selection and confirmation of hybrids with the GFP meter

GFP fluorescence intensity measured in the sampled leaves increased as plant aged during the vegetative growth of these hybrids (Table **1**). There was a significant difference of GFP values measured between field and greenhouse ($F_{1,2} = 7.220$, $p < 0.01$), and GFP at various leaf stages was different ($F_{2,33} = 66.195$, $p < 0.01$) for hybrids obtained between transgenic OSR and conventional crop. GFP fluorescence in hybrids between transgenic OSR and wild mustard was statistically significantly higher ($F_{1,2} = 16.346$, $p < 0.01$) compared with hybrids between transgenic OSR and conventional rapeseed crop in the greenhouse at corresponding leaf stages (Table **1**), and the difference at different developmental stages was significant ($F_{2,21} = 44.515$, $p < 0.01$).

Bt protein concentration in hybrids

Bt protein concentration increased in leaves of all hybrids as they aged (Table **2**). A significant difference in mean concentrations was found between field and greenhouse experiments ($F_{1,2} = 14.085$, $p < 0.01$), the difference at various leaf stages was significant ($F_{2,33} = 21.090$, $p < 0.01$) for hybrids obtained

Table 1 The ranges of GFP fluorescence intensity (arbitrary units) at various developmental stages of the hybrids between transgenic oilseed rape and conventional crop variety Xiangyou no. 15 or wild mustard in the greenhouse or field, mean ± SE is given in parenthesis

Developmental stages	Field measurements	Greenhouse measurement	
	Hybrids of Xiangyou	Hybrids of Xiangyou	Hybrids of wild mustard
Two-leaf	423 – 213 (271 ± 38.4)	194 – 101 (134 ± 11.0)	314 – 283 (297 ± 3.9)
Four-leaf	582 – 366 (513 ± 39.1)	269 – 143 (179 ± 14.4)	563 – 310 (414 ± 32.5)
Six-leaf	745 – 568 (640 ± 35.5)	1 098 – 508 (804.6 ± 77.7)	1 090 – 477 (858 ± 69.6)

Table 2 The ranges of Bt protein concentration (ng/g fresh leaf weight) at various developmental stages of the hybrids between transgenic oilseed rape and conventional crop variety Xiangyou no. 15 or wild mustard in the greenhouse or field, mean ± SE is given in parenthesis

Developmental stages	Field measurements	Greenhouse measurement	
	Hybrids of Xiangyou	Hybrids of Xiangyou	Hybrids of wild mustard
Two-leaf	177 – 39 (97 ± 23.6)	120 – 53 (79 ± 8.8)	206 – 107 (159 ± 11.2)
Four-leaf	315 – 144 (239 ± 28.7)	257 – 65 (137 ± 26.6)	514 – 188 (282 ± 40.5)
Six-leaf	653 – 220 (428 ± 77.2)	416 – 96 (215.5 ± 36.2)	725 – 300 (496 ± 44.7)

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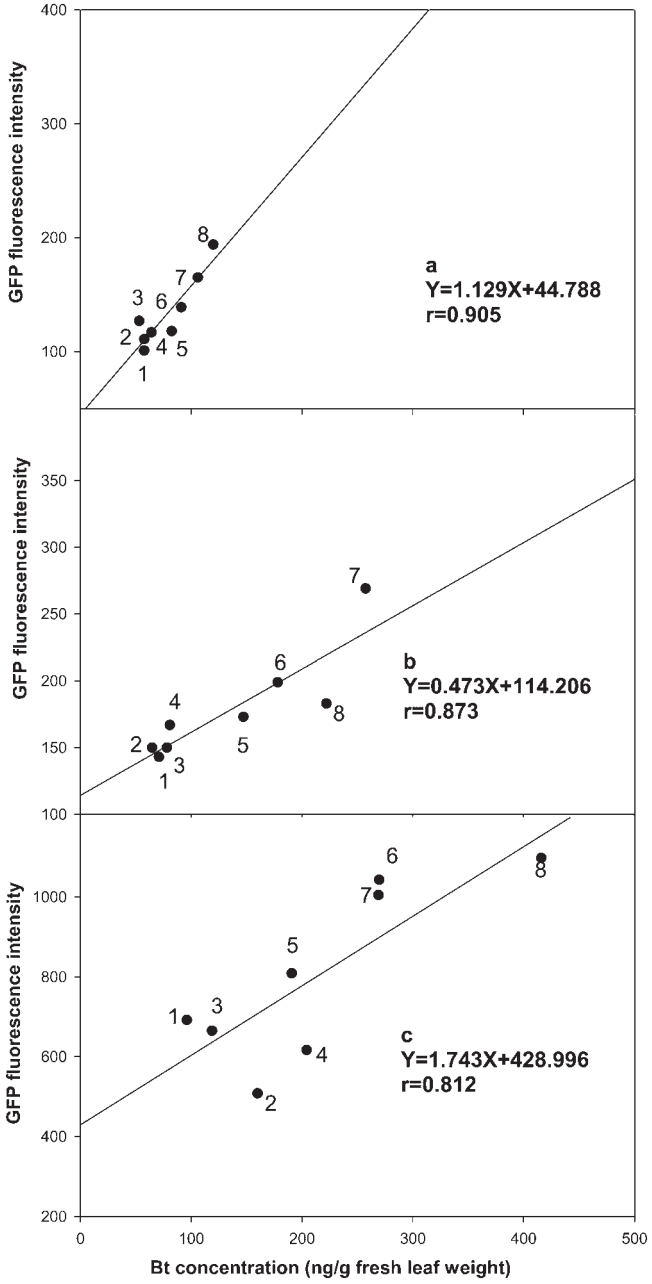

Fig. 4 GFP fluorescence intensity (arbitrary units) of transgenic hybrids produced between transgenic oilseed rape and conventional Chinese rapeseed variety (Xiangyou no. 15), measured in the greenhouse, is correlated with the concentration of Bt protein at two-leaf (**a**), four-leaf (**b**), and six-leaf (**c**) stages. The numbers (1 – 8) in these figures indicate individual hybrid plants.

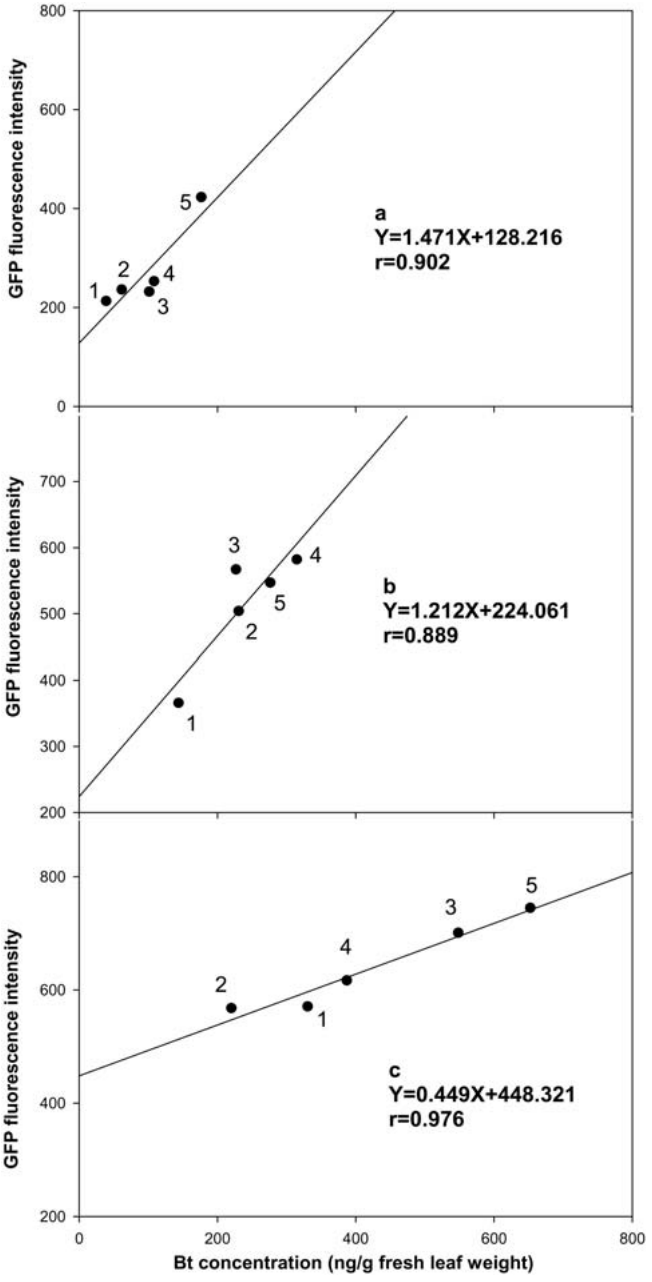

Fig. 5 GFP fluorescence intensity (arbitrary units) of transgenic hybrids produced between transgenic oilseed rape and conventional Chinese rapeseed variety (Xiangyou no. 15), measured in the field, is correlated with the concentration of Bt protein at two-leaf (**a**), four-leaf (**b**), and six-leaf (**c**) stages. The numbers (1 – 5) in these figures indicate individual hybrid plants.

between transgenic OSR and Chinese OSR. There was also a significant difference of Bt protein concentration in transgenic OSR-wild mustard hybrids at different developmental stages ($F_{2,21} = 23.323$, $p < 0.01$).

Correlation between Bt and GFP expression

Hybrids grown in the greenhouse had a significant correlation (Fig. **4**) with GFP and Bt expression at the two-leaf stage ($F_{1,6} = 27.140$, $p < 0.01$), four-leaf stage ($F_{1,6} = 19.190$, $p < 0.01$), and six-leaf stage ($F_{1,6} = 11.57$, $p < 0.05$). Thus, the Bt concentration in hybrids between transgenic OSR and Xiangyou no. 15 could be estimated by the correlation between GFP fluorescence and Bt protein. For the hybrids in the field, GFP fluorescence was also significantly associated with Bt content in the leaf at all developmental stages (Fig. **5**) of two-leaf ($F_{1,3} = 13.04$, $p < 0.05$), four-leaf ($F_{1,3} = 11.36$, $p < 0.05$), and six-leaf ($F_{1,3} = 61.29$, $p < 0.01$) plants. The correlations (r) in these hybrids were between 0.812 and 0.976, where the highest value appeared at the six-leaf stage measured in the field, while the

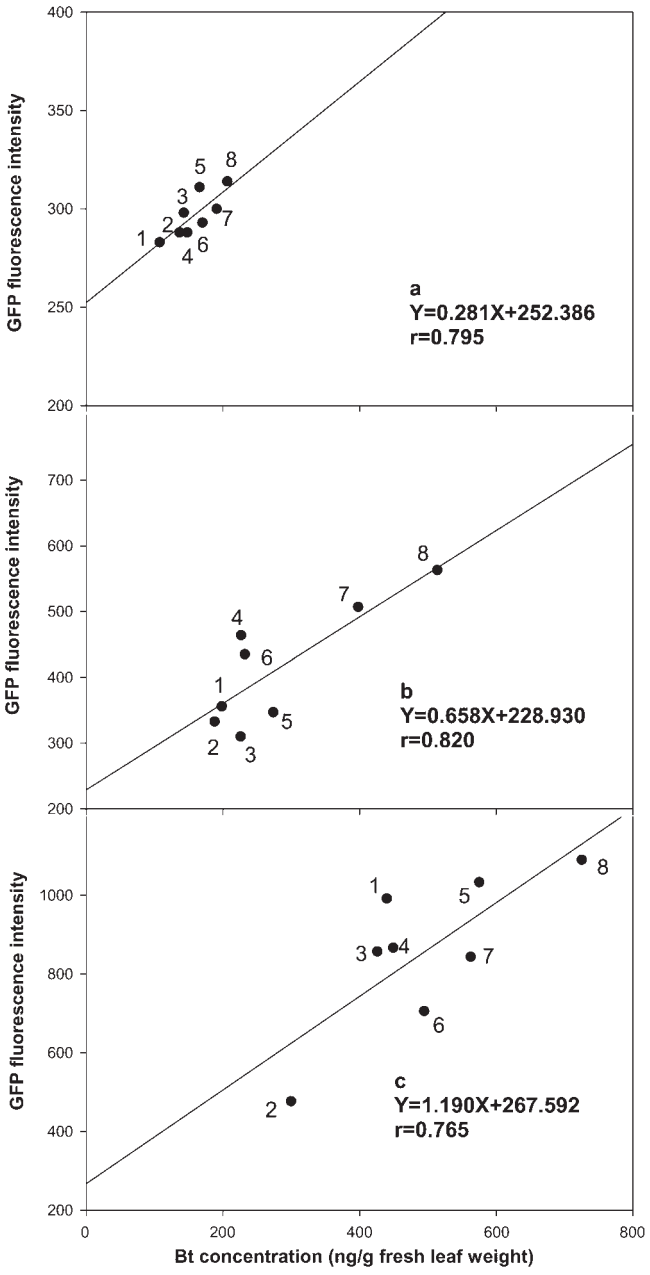

Fig. 6 GFP fluorescence intensity (arbitrary units) of transgenic hybrids produced between transgenic oilseed rape and wild mustard, measured in a greenhouse, is correlated with the concentration of Bt protein at two-leaf (**a**), four-leaf (**b**), and six-leaf (**c**) stages. Numbers (1 – 8) in these figures indicate individual hybrid plants.

lowest value appeared at the same stage in the greenhouse. This again highlighted differences in measurements in different environments.

The correlation between GFP fluorescence and Bt protein in hybrids between transgenic OSR and wild mustard was also significant at all stages (Fig. **6**): the correlation (r) was 0.795 at two-leaf stage ($F_{1,6} = 10.31$, $p < 0.05$), 0.820 at four-leaf stage ($F_{1,6} = 12.32$, $p < 0.05$), and 0.765 at six-leaf stage ($F_{1,6} = 8.46$, $p < 0.05$). The Bt concentration in hybrids of wild mustard could be calculated from GFP fluorescence observations.

Discussion

Halfhill et al. (2003) reported a decrease in GFP fluorescence as leaves aged from four-leaf stage and GFP intensity ranged from 764 to 1175 without subtracting the negative control at the four-leaf and six-leaf stages in transgenic oilseed rape. However, a slight increase of fluorescence intensity was observed in Figure 1 in their paper at the uppermost position of the plants from four-leaf to six-leaf stages. Here we reported an increase of GFP fluorescence intensity from 2-leaf stage to four-leaf stage and six-leaf stage in the hybrids formed between transgenic OSR and its relatives and its value in arbitrary units ranged from 143 to 1098 after subtracting the negative controls at the last two leaf-stages, at which stages the two studies could only be compared. The high variation in GFP fluorescence could be hybrid-specific (Halfhill et al., 2001; Richards et al., 2003).

In addition, Halfhill et al. (2003) showed that the expression of GFP protein was positively related to total soluble protein in transgenic OSR. In this study, increased total soluble protein was observed as GFP fluorescence intensity increased during the two-, four-, and six-leaf stages in hybrids (data not shown), while increased GFP fluorescence intensity was still associated with increased Bt Cry1Ac protein that should have an effect on target insects (Wei et al., 2005). The results show no conflict with current literature but extend current findings to demonstrate that co-expression of transgenes occurs in varied genetic background.

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