Mapping of Quantitative Trait Loci Involved in Ornamental Traits in *Alstroemeria*

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Abstract. An F1 population, derived from an intraspecific cross between two *Alstroemeria aurea* accessions, was used to map quantitative trait loci (QTL) involved in ornamental and morphological characteristics. One QTL for leaf length was mapped on linkage group three of both parents near marker E+ACCT/M+CGCA-I165 explaining 20% and 14.8% phenotypic variation. Two putative QTL were detected on leaf width on A002-3 and A002-6. One QTL and three putative QTL, involved in the leaf length/width ratio were identified accounting for 46.7% of the phenotypic variance in total. Significant interaction was observed between two QTL S+AC/M+ACT-I112 and S+AC/M+AGA-I465 in a two-way analysis of variance (ANOVA). For the main color of the flower one QTL and putative QTL accounted for up to 60% of phenotypic variance suggesting simple genetic control of flower color. A two-way ANOVA of these QTL suggested an epistatic interaction. A QTL was detected for color of the inner side of outer lateral tepal with 26.5% of the phenotypic variance explained. This QTL was also associated with main color of the flower just below the 95% threshold value. Two QTL were detected with the Kruskal-Wallis test for the tip color of inner lateral tepal near QTL for other flower color traits. Consequently flower color traits were significantly correlated. A QTL associated with the Kruskal-Wallis test for the stripe width of the inner lateral tepal. One putative QTL was detected for the construction of linkage maps (Van Heusden et al., 2000) that has an equally “large” genome (30,850–33,500 Mbase) (Bennett et al., 1998). Recently, the AFLP marker technique was used for a biodiversity study of *Alstroemeria* species (Han et al., 2001) and for the construction of an *A. aurea* linkage map (Han et al., 2001).

*Alstroemeria aurea* is commonly used in cultivar development as one of the parents. If prior knowledge of the linkage relationships between marker loci and important ornamental characteristics of *A. aurea* were available, marker-assisted selection could be performed in seedling stages and used to eliminate undesirable individuals from progeny populations. Linkage relationships between molecular markers and morphological traits have been studied in many crop plants such as potato (*Solanum tuberosum* L.) (Van Eck et al., 1993; Van Eck et al., 1994a), wheat (*Triticum sp.*)(Kato et al., 1999), sunflower (*Helianthus annuus* L.) (Gentzibittel et al., 1999), tomato (*Lycopersicum esculentum* Mill.) (Grandillo et al., 1999), barley (*Hordeum vulgare* L.) (Zhu et al., 1999), and cotton (*Gossypium sp.*) (Jiang et al., 2000). In ornamentals, flower traits such as petal number and flower color have recently been mapped for the first time in rose by using RAPD and AFLP markers (Debener and Mattiesch, 1999). No similar study has been reported in *Alstroemeria* before this paper.

The recent construction of the first molecular linkage map in *A. aurea* (accession A002 x accession A003) using AFLP markers (Han et al., 2001) facilitated the first quantitative trait loci (QTL) analysis in *A. aurea*. We present data on 14 traits that are important in ornamentals. Subsequently, the inheritance of these traits has been selected by QTL analysis, to identify genetic loci responsible for the morphological variation in *A. aurea* accessions.

Materials and Methods

Plant material. A population of 134 F1 individuals was obtained from an intraspecific cross of the *A. aurea* accessions A002 x A003 (Han et al., 2001). The genotypes A002 and A003, which are commonly used progenitor lines from breeding companies, showed distinctive morphological differences such as in flower color; red and yellow respectively. Parents and the F1 descendants were grown under the same greenhouse conditions.

Phenotypic analyses. The 134 individual F1 plants were scored for 14 morphological and ornamental traits (Table 1). The majority of these traits were chosen on the basis of the Union internationale pour la protection des obtentions vegetales (UPOV) list of cultivar descriptors (UPOV, 1987). UPOV character.

| Trait code | Trait name | Trait values of parental genotypes A002; A003 | UPOV character no. |
|------------|------------|-----------------------------------------------|-------------------|
| L1         | Leaf length| 9.99; 12.84 cm                                 | 4                 |
| L2         | Leaf width | 1.77; 1.65 cm                                  | 5                 |
| L3         | Ratio of leaf length and width                  | 5.67; 7.87        | 6                 |
| L4         | Length of leaf petiole                          | 2.05; 2.26 cm     | 7                 |
| C1         | Main color of complete flowers                  | Red; Yellow       | 11                |
| C2         | Color of inner side of outer lateral tepal      | Red; Yellow       | 16                |
| C3         | Tip color of inner lateral tepal                | Red; Yellow       | 20*               |
| F1         | Flower size                                      | 3.92; 4.52 cm     | 12                |
| F2         | Flower openness*                                 | Open; Close       | 13                |
| F3         | Number of flowering stems                        | Not determined for the parents                  |
| F4         | First date of flowering                          | 4 June; 10 April  |                   |
| F5         | Last date of flowering                           | 28 June; 15 June  |                   |
| H6         | Flowering period in days                         | 24 d; 66 d       |                   |
| S1         | Stripe width of inner lateral tepal             | 0.83; 0.73 mm     | 22                |

*Trait analyzed only by Kruskal-Wallis test.

1Modified UPOV character. Not middle zone, but tip color of the inner lateral tepal was studied.
Genotyping and data analysis. AFLP segregation and marker order on A. aurea linkage maps, used in this study for QTL identification, have been described previously (Han et al., 2001). QTL detection was based on separate parental data sets and non-integrated maps of A002 and A003. Each of the parental data sets does include some allelic bridge markers, which segregate in a 3:1 ratio. However, the position of these 3:1 markers on the A002 and A003 linkage groups cannot be accurately determined (Maliepaard et al., 1997). Therefore, the marker sequence on the integrated map will be also highly ambiguous, even if the marker order within each parental map would be fixed. Moreover, not all linkage groups could be integrated with a homologous group from the other parent. A more accurate integration of the A002 and A003 linkage groups would require at least codominant markers, and preferably allowing full classification such as SSR or RFLP markers. These are not available in Alstroemeria.

Average trait values and correlation coefficients between traits were calculated to det-
Fig. 1. Histograms of genotype estimates for traits relating to morphological traits scored on individuals from the segregating population derived from 'A002 × A003'. Mean parental values are indicated with lines. The phenotypic values are transformed by square root.
QTL detected by E+ACCC/M+CGCG-93 on the tip color of inner lateral tepal (C3). The threshold value (LOD = 2.9) at a LOD of 2.65, which is just below the 95% value.

Several aspects of productivity have been studied. Productivity of cut flowers can be represented by the number of flowering stems. Therefore, the number of flowering stems was counted during the flowering season. However, out of the 134 genotypes 49 did not produce any flowering stem, which was not of a genotype that did not flower could be indicated with a ‘zero’ or it could be a missing value. Both options have been tested in QTL analysis and both options resulted in putative QTL. However, the putative QTL observed by interval analysis were not significant with the Kruskal-Wallis test, and vice versa. Moreover, none of these putative QTL was detected for both options. A third option to analyze this trait is to treat it as a binary character: Yes or No flowering. This did not result in the detection of any genetic locus. In view of these contradictory results, we do not feel confident to mention any of the putative QTL.

Another aspect of productivity, which is highly confounded with the number of flowering stems, is the flowering period. When more stems are produced, the period will also be longer. Three aspects of flowering period were studied: 1) first date of flowering, representing earliness of the crop; 2) last date of flowering, representing the joint effect of number of stems and lateness of the first flowering period, which is the interval between the first two traits. A putative QTL for first date of flowering (F4) was detected with marker E+AATC/M+CGCG-1112 on A002-7 with 7.7% of the phenotypic variance explained. A putative QTL marker for last date of flowering (F5) was marker E+ACCA/M+CGCC-222 on A003-1 with 13.3% of the phenotypic variance explained. No QTL were detected for duration of flowering period.

Discussion

In the offspring of the cross between *Alstroemeria* accessions A002 x A003, a large number of morphologically and ecologically important traits segregated, resulting in highly diverse offspring genotypes. This rich genetic variation was studied by QTL analysis using AFLP markers mapped on separate parental linkage groups of A002 and A003. The range of phenotypes observed in the offspring is described by the frequency distributions of the trait values in Fig. 1, together with the parental trait values. For the morphological traits of the leaves/length, width, shape (length : width ratio) and leaf petiole length, flower size, stripe width, and three aspects of flowering period, phenotypic values have been ob-

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**Table 2. Position and nearest markers of QTL (bold) and putative QTL detected for each morphological, ornamental, or both traits, as detected by interval mapping or Kruskal-Wallis test.**

| Trait code | Linkage group | LOD peak position (cM) | Nearest marker | LOD (%) | 95% LOD | 99% LOD |
|------------|---------------|------------------------|----------------|---------|---------|---------|
| L1 A002-3  | A002-1        | 28.8                   | E+ACCT/M+CGCA-1165 | 20.0    | 4.81    | 2.9     |
| L1 A002-3  | A002-1        | 28.8                   | E+ACCT/M+CGCA-1165 | 20.0    | 4.81    | 2.9     |
| L2 A002-6  | A002-3        | 39.0                   | E+ACCT/M+CGCA-1165 | 14.8    | 3.63    | 3.0     |
| L2 A002-6  | A002-3        | 39.0                   | E+ACCT/M+CGCA-1165 | 14.8    | 3.63    | 3.0     |
| L3 A002-4  | A002-1        | 13.9                   | S+AC/M+ACT-193    | 12.8    | 2.59    | 2.4     |
| L3 A002-4  | A002-1        | 13.9                   | S+AC/M+ACT-193    | 12.8    | 2.59    | 2.4     |
| L1 A003-9  | A003-1        | 23.5                   | S+AC/M+ACT-193    | 11.2    | 2.79    | 2.4     |
| L1 A003-9  | A003-1        | 23.5                   | S+AC/M+ACT-193    | 11.2    | 2.79    | 2.4     |
| C1 A003-1  | A003-1        | 24.5                   | E+AATC/M+CGCG-1168| 24.5    | 3.35    | 3.3     |
| C1 A003-1  | A003-1        | 24.5                   | E+AATC/M+CGCG-1168| 24.5    | 3.35    | 3.3     |
| C2 A003-9  | A003-2        | 26.5                   | E+AATT/M+CGAT-222 | 26.5    | 2.70    | 2.7     |
| C2 A003-9  | A003-2        | 26.5                   | E+AATT/M+CGAT-222 | 26.5    | 2.70    | 2.7     |
| C3 A003-3  | A003-3        | 47.1                   | E+AATC/M+CGCG-1168| 31.6    | 3.85    | 3.0     |
| C3 A003-3  | A003-3        | 47.1                   | E+AATC/M+CGCG-1168| 31.6    | 3.85    | 3.0     |
| F1 A002-5  | A002-2        | 9.1                    | E+ACCC/M+CGCT-193 | 15.5    | 2.97    | 2.7     |
| F1 A002-5  | A002-2        | 9.1                    | E+ACCC/M+CGCT-193 | 15.5    | 2.97    | 2.7     |
| S1 A002-2  | A002-2        | 47.1                   | S+AG/M+ATT-197    | 15.5    | 2.97    | 2.7     |
Fig. 2. QTL (solid bars or **) and putative QTL (hatched bars or *) associated with the traits are depicted along the linkage groups of the A. aurea map. QTL detected by interval mapping are indicated by a bar. The length of the bar equals the 1 LOD support interval. QTL detected by Kruskal-Wallis are indicated by one or two asterisk attached to the marker position. (Fig. 2 continues on next page.)
Fig. 2. continued.
served in the progeny that go beyond the parental values. These transgressive segregations are typical for the offspring of non-inbred species, and result from heterozygosity in the parents for QTL alleles with positive as well as negative effects. In the case of flower color traits (C1, C1, and C3) the parents showed extreme phenotypes, with offspring phenotypes ranging between the parental extremes. Nevertheless, extreme parental phenotypes did not result from fixation (homozygosity) at genes involved in yellow and red flower pigmentation. The very large genome size of Alstroemeria makes it easy to develop such single locus markers in the genome. In principle true, unfortunately in practice the homozygosity at these loci would have resulted in a uniform F1. On the contrary, heterozygosity was present both in parent A002 and A003, as QTL involved in flower color have been mapped on A002 and A003. Although the number of loci involved in flower color does not seem large, a simple genetic model could not be deduced. On the basis of our results, dominance relations between red and yellow could not be inferred. Some of the QTL were involved in more than one trait. For example, the QTL at E+ACCT/ M+Cgca-A1165 on linkage group A002-3 was involved in L1 and L2, which suggests a pleiotropic effect of a single QTL. Similarly, on A003-9 two closely linked markers E+Att/M+Gcat-222 and E+Att/M+Gcat-248 each map a QTL involved in color traits C1 and C3 respectively, also suggesting the presence of a single QTL with pleiotropic effect. The presence of two different QTL with close linkage is not very likely in these cases, because of the high related leaf and color phenotypes, respectively.

In this study, QTL for ornamental characters have been mapped on Alstroemeria linkage maps using AFLP markers mapped on noninverted parental linkage groups of A002 and A003. It was observed that many of the QTL involved are high LOD values, or did not reach highly significant K-values in the Kruskal-Wallis test. It was also observed that QTL detected by interval mapping were not often confirmed by the Kruskal-Wallis test and vice versa. One of the obvious explanations is that our approach is not good. The phenotype is the joint result of the allele(s) derived from A002 and A003, and therefore, QTL should be mapped with the integrated map instead of the separate parental maps. Possibly some QTL would have reached much higher LOD scores when data from an integrated map were available, and possibly more QTL would have been detected. Although this is true, unfortunately in practice this is not easily achieved. A large amount of codominant and multiallelic markers such as SSR or RFLP, resulting in a 1:1:1:1 segregation is required to obtain full classification of the offspring, and to obtain a good integrated map. In Alstroemeria, such markers have not been developed, and moreover, it will not be easy to develop such single locus markers in view of the limitations posed by the very large genome size of Alstroemeria. The signal detection threshold for RFLP is at picogram amounts of DNA on a Southern Blot, but before reaching such amounts of target sequence, diluted in the huge genome, then probably the binding capacity of such a blot has been exceeded. The reproducibility of single locus PCR based markers such as SSR may be affected in large genome species. As a consequence the choice of AFLP over RFLP and SSR in this study was based on the high multiplex ratio, reproducibility and low development time/costs of AFLP.

Moreover, the development of SSR via the identification or enrichment of simple sequence repeat in DNA sequences is also hampered by the large genome size. King and Maliepaard et al. (2000) found a highly related leaf and color phenotypes, respectively. In this way, information of several 1:1 and/or 3:1 markers are used to compensate for the missing information, and allow to reach the power of a 1:1:1:1 segregating marker set. However, this ambiguity in marker order in the integrated map will have such a negative effect on these estimated genotypes at a certain map position, that the power of interval mapping and the Kruskal-Wallis test will be affected severely. In that case the gain of power by integration of the map is lost by the inaccuracies in marker order.

Another explanation for the poor significance of the QTL could be found in the (effective) population size. Although 134 offspring genotypes have been typed and phenotyped, this number has not always been exceeded. The reproducibility of RFLP marker sets, Maliepaard et al. (1997) have described the poor accuracy of the position of these 3:1 markers relative to the position of the 1:1 markers. The MapQTL option for cross-pollinator AFLP data was used instead of the MapQTL option for cross-pollinator AFLP data was used in order to obtain an overall significance level of 0.005. To control the significance level for interval mapping we used the permutation test (Churchill and Doerge, 1994), implemented in MapQTL®. Due to the massive amount of calculation, however, Van Ooijen (1999) described an alternative method of getting the significance threshold without the permutation test. However, current computer capacity allowed us to calculate significance thresholds for each trait, taking into account the quality of the map and the trait values. In this study significance LOD thresholds ranging between LOD = 3.2 – LOD = 4.9 were obtained at the 99% confidence level. At this level we detected a total of 3 QTL on 3 linkage groups, and additionally 11 QTL on nine linkage groups at the 95% confidence level.

**Literature Cited**

Bennett, M.D., A.V. Cox, and I.J. Leitch. 1998. Angiosperm DNA C-values database. http://www.rbgkew.org.uk/cval/database1.html.

Bouton, J.H., E.J. Boose, and M.A. Jackson. 1997. Nuclear DNA content in twelve species of Alstroemeria L. and some of their hybrids. Ann. Bot. 79:343–353.

Byrne, M., J.C. Murrell, J.V. Owen, P. Kriedemann, E.R. Williams, and G.E. Moran 1997. Identification and mode of action of quantitative trait loci affecting seedling height and leaf area in Eucalyptus nitens. Theor. Appl. Genet. 94:674–681.

Churchill, G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138:963–971.

Debener, T. and L. Mattiessch. 1999. Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor. Appl. Genet. 99:891–899.

Gentzbittel, L., E. Mestries, S. Mouzeyar, F. Mazeyrat, S. Badaoui, F. Vear, D.T. de Labrouhe, and P. Nicolas. 1999. A composite map of expressed sequences and phenotypic traits of the sunflower (Helianthus annuus L.) genome. Theor. Appl. Genet. 99:218–234.

Grandel, S., H.M. Ku, S.D. Tanksey. 1999. Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor. Appl. Genet. 99:978–987.

Grattapaglia, D., F.L. Bertolucci, R.R. Sederoff. 1995. Genetic mapping of QTLs controlling vegetative propagation in Eucalyptus grandis and E. urophylla using a pseudo-testcross strategy and RAPD markers. Theor. Appl. Genet. 90:933–947.
Groover, A., M. Devey, T. Fiddler, J. Lee, R. Megraw, T. Mitchel-Olks, B. Sherman, S. Vujcic, C. Williams, and D. Neale. 1994. Identification of quantitative trait loci influencing wood specific gravity in an outbred pedigree of loblolly pine. Genetics 138:1293–1300.

Han, T.H., H.J. Van Eck, M.J. De Jeu, and E. Jacobsen. 1999. Optimization of AFLP fingerprinting of organisms with a large genome size: A study on Alstroemeria spp. Theor. Appl. Genet. 98:465–471.

Han, T.H., M.J. De Jeu, H. Van Eck, and E. Jacobsen. 2000. Genetic diversity of Chilean and Brazilian Alstroemeria species assessed by AFLP analysis. Heredity 84:564–569.

Jiang, C., R.J. Wright, S.S. Woo, T.A. DelMonte, and A.H. Paterson. 2000. QTL analysis of leaf morphology in tetraploid Gossypium (cotton). Theor. Appl. Genet. 100:409–418.

Kato, K., H. Miura, and S. Sawada. 1999. QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. Theor. Appl. Genet. 98:472–477.

King, G.J., C. Maliepaard, J.R. Lynn, F.H. Alston, C.E. Durel, K.M. Evans, B. Griffon, F. Laurens, A.G. Manganaris, E. Schrevens, and S. Tartarini. 2000. Quantitative genetic analysis and comparison of physical and sensory descriptors relating to fruit flesh firmness in apple (Malus pumila Mill). Theor. Appl. Genet. 100:1074–1084.

Kruglyak, L., and E.S. Lander. 1995. A nonparametric approach for mapping quantitative trait loci. Genetics 139:1421–1428.

Lander, E.S. and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–190.

Maliepaard, C., J. Jansen, and J.W. Van Ooijen. 1997. Linkage analysis in a full-sib family of an outbreeding plant species: overview and consequences for applications. Genet. Res. 70:237–250.

Union internationale pour la protection des obtentions vegetales (UPOV). 1987. Guidelines for the conduct of tests for distinctness, homogeneity and stability. TG29/6 (revision of TG29/5) http://www.upov.org.

Van Heusden, A.W., J.W. Van Ooijen, R. Vrielink-van Ginkel, W.H.J. Verbeek, W.A. Wietema, and C. Kik. 2000. A genetic map of an interspecific cross in Allium based on amplified fragment length polymorphism (AFLP®) markers. Theor. Appl. Genet. 100:409–418.

Van Eck H.J., J.M.E. Jacobs, P.M.M.M. Van den Berg, W.J. Stiekema, E. Jacobsen. 1994a. The inheritance of anthocyanin pigmentation in potato (Solanum tuberosum L.) and mapping of tuber skin colour loci using RFLPs. Heredity 73:410–421.

Van Eck H.J., J.M. Jacobs, P. Stam, J. Ton, W.J. Stiekema, and E. Jacobsen. 1994b. Multiple alleles for tuber shape in diploid potato detected by qualitative and quantitative genetic analysis using RFLPs. Genetics 137:303–309.

Van Ooijen J.W. 1999. LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83:613–624.

Van Ooijen J.W. 2000. MapQTL® Version 4.0: User friendly power in QTL mapping. Plant Research International, http://www.plant.wageningenur.nl/products/mapping/MapQTL/mqintro.htm.

Voorrips, R.E. 2000. MapChart version 1.4: Windows software for the graphical presentation of linkage maps and QTLs. Plant Res. Intl., Wageningen.

Zhu H., L. Gilchrist, P. Hayes, A. Kleinbogs, D. Kudrna, Z. Liu, L. Prom, B. Stefferson, T. Toojinda, and H. Vivar. 1999. Does function follow form? Principal QTL for Fusarium head blight (FHB) resistance are coincident with QTL for inflorescence traits and plant height in a doubled-haploid population of barley. Theor. Appl. Genet. 99:1221–1232.