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RESEARCH ARTICLE

Mapping QTLs associated with chloride accumulation in leaves of oriental tobacco (*Nicotiana tabacum* L.) using $F_{2:3}$ population of Basma Seres 31 × SPT 406 cross

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ABSTRACT: Chloride is considered as the most important micronutrient in tobacco production. But excessive amounts of chloride accumulation in leaves of tobacco have many adverse effects on the tobacco quality, such as burning capacity. Identification of quantitative trait loci (QTL) involved in chloride accumulation would be beneficial for the improvement of tobacco quality. The objective of this study was to identify genomic regions associated with chloride accumulation by using a mapping population consists of 225 F2:3 families derived from hybridization between 'Basma Seres 31' and 'SPT 406' lines. Linkage map was constructed with 23 microsatellite (SSR) and 29 inter simple sequence repeat (ISSR) polymorphic markers which covered 570.8 cM of the tobacco genome. Thirty-four of these polymorphic markers were mapped to 7 linkage groups. Distance between two adjacent markers was 17.3 cM. Composite interval mapping (CIM) was used to identify QTLs controlling chloride accumulation. One QTL for chloride accumulation was identified on linkage group 3. The percentage of phenotypic variance (R^2) explained by this QTL was 12.7%. A significant association was not found between ISSR markers and chloride accumulation. The outcome of present effort can be a basis for marker aided selection (MAS) in tobacco breeding programs.

KEYWORDS: Chloride accumulation, Composite interval mapping, Linkage map, Oriental tobacco

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is the most important agricultural crop plant for the economy of many countries [21]. *Nicotiana tabacum* is an amphidiploid plant arisen by natural hybridization of *N. sylvestris* and *N. tomentosiformis* species [19; 27]. Tobacco is at the high end of genome sizes (4.5 Gbp) in the Solanaceae [2] and contains a large proportion of repetitive sequences [26, 44]. In Iran, tobacco is one of the most important industrial crops with more than six thousand acres under cultivation and an annual production of nearly 10 thousand tons (wet weight). Area under cultivation of tobacco in the world is 4.8 million hectares with annual production of 7.1 million tons. Tobacco is also one of the most important model systems in systematic, plant biology, genetics and plant biotechnology [28] and highly promising for the production of commercially important substances such as medical drugs and vaccines [6, 7]. Numerous types of tobacco are defined by different criteria such as morphological and biochemical characteristics [30, 31]. Turkish or oriental tobacco is a sun-cured, aromatic, small-leafed type which is grown in Turkey, Iran, Bulgaria, Greece, Lebanon and the Republic of Macedonia. This type has a much milder flavor and contains less nicotine and fewer carcinogens than other

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types [14]. The leaf of tobacco contains mineral matter, organic compounds and 85–90% water. Among mineral nutrients, chloride (Cl^-) is an essential micronutrient for tobacco plantation. Many studies cleared that small amount of chloride (below 1.5%) is effective in increasing tobacco yields and improvement of burning quality [9]. Excessive amounts of chloride have many adverse effects on the quality of tobacco leaves, such as poor burning capacity, lacking in toughness and elasticity, muddy appearance, undesirable odor [18]. High chloride content (over 2.0%) results incombustible tobacco leaves [13]. According to studies, with the increase of chloride, both hygroscopicity and burning quality decrease [23]. However, apparently the burning quality is also largely influenced by the potassium content [10] and leaf position [5]. Chloride reduces the content of potassium salts of organic acids which are useful for burning [23]. Therefore, how to increase the potassium content or reduce chloride content was crucial for improving the quality of tobacco leaves. Great genetic variations have been reported for chloride accumulation in oriental tobacco germplasm [13]. Both additive and non-additive genetic components control chloride accumulation in tobacco leaves [12].

Normally agronomically important characters exhibit polygenic inheritance. Identification and localization of quantitative trait loci (QTL) controlling characters can simplify the selection process via marker-aided selection (MAS) in plant breeding activities [35; 39]. Some genetic linkage maps were constructed for tobacco using molecular marker such as restriction fragment length polymorphism (RFLP) [17], conserved ortholog sequences [36] and simple sequence repeat (SSR) markers [4]. In a study, a total of 184 amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), sequence specific amplified polymorphism (SSAP), and sequence characterized amplified region (SCAR) were used to construct a partial genetic map in tobacco [25]. In another study, using a partial genetic linkage map comprising 18 groups, QTL analysis was conducted for a total of 59 traits related to diverse agronomic, leaf quality, chemical composition, and smoke characteristics [25]. The QTLs responsible for different characters such as the amount of nicotine, leaf potassium content and sugar [8; 25], resistance to leaf spot disease [34] and chloride concentration [22] have been identified.

The chloride content is considered as a major factor determining the quality of tobacco leaves. In the present study, we aimed to identify QTLs associated with chloride accumulation in oriental tobacco leaves by using 225 F2:3 families derived from the cross between Basma Seres 31 × SPT 406 lines based on SSR and ISSR markers. The outcome of the present study can potentially speed up the breeding activities in oriental tobacco trying to producing new cultivars with low chloride accumulation.

MATERIALS AND METHODS

Plant materials and measurement of chloride accumulation

In this study, a population of 225 F2:3 families were derived from the cross between 'Basma Seres 31' as a maternal line with high chloride accumulation and 'SPT 406' as a paternal line with low chloride accumulation and self-pollination of F2 progenies. A total of 225 families along with the parental lines were evaluated in 15 × 15 sample lattice design with two replicates in Urmia Tobacco Research Center with 44.58° longitude and 37.34° latitude and an altitude of 1300 meters from sea level. Each plot was consisted of three rows. Inter-row and within-row spacing were 65 cm and 20 cm, respectively (eight thousand plants per hectare). Agricultural land preparation including deep plowing and relative deep plowing was conducted in autumn and spring, respectively. After preparing the mainland and before plantlet transplanting, Eradican herbicide, pure nitrogen, phosphor, and potash in the amount of 4l ha^{-1}, 52 kg ha^{-1}, 96 kg ha^{-1}, and 150 kg ha^{-1}, respectively were broadcasted and mixed with soil by disc. Farm irrigation was performed when 80% of soil moisture was drained.

Three sun-cured leaves from upper, middle and lower regions of each plant were used to determine chloride content. Concentration of chloride was determined following the method described by [13] and calculated according to Equation 1:

Equation 1:
$$Cl = \frac{(A-B).f.35.3}{W\dfrac{(100-M)}{100}} \times 100$$

Where A is the mount of AgNO3 used for tobacco sample, B is the mount of AgNO3 used for blank sample, W is the tobacco weight, M is the percentage of leaf humidity and f is the normality of AgNO3.

DNA extraction, SSR and ISSR reaction

Total genomic DNA was extracted from the leaves of parental lines and F2 individuals as described by

Dellaporta et al., [15]. An initial analysis of polymorphism was performed in two parental lines. Polymerase chain reaction (PCR) was performed using 162 pairs of SSR and 80 ISSR markers according to [16] and [40], respectively. A total of 52 markers were polymorphic between the parents. These candidate markers were then analyzed on F2 individuals.

QTL mapping

The Carthagene software [20] with Kosambi mapping function was used for linkage analysis. A LOD score of 3.0 was used as the threshold to declare the presence of QTLs. The maximum distance between markers was 50 cM. The Windows QTL Carthographer [3] was used to identify QTLs controlling concentration of chloride based on the composite interval mapping (CIM) [41, 42] methods. The LOD (log10 likelihood ratio: likelihood that the effect occurs by linkage/ likelihood that the effect occurs by chance) score was determined by permutation testing (n=1,000 permutations) [11].

RESULTS and DISCUSSION

Phenotypic data

To identify the loci controlling chloride accumulation in oriental tobacco leaves, a mapping population including 225 F2:3 families were created by hybridization of Basma Seres 31 and SPT 406. Basma Seres 31 showed 4-fold higher chloride accumulation in leaves in comparison to SPT 406 genotype ($P<0.01$) (Table 1). F2:3 families showed continuous frequency distribution pattern for chloride accumulation indicating quantitative inheritance behaviour. Some F2:3 families showed transgressive segregation either in positive or negative direction for Cl^- accumulation in leaves (Figure 1). Similar result was achieved by Hatami Maleki et al. [22] in the F2 population of same cross. Several studies point to the action of complementary genes as the primary cause of transgression, although overdominance and epistasis also contribute [32].

Table 1. Comparison of values related to chloride concentration between parents of mapping population.

Trait	Genotype	Mean	SD	t-value
Chloride	'Basma Seres 31'	2.08	0.27	-5.65**
	'SPT 406'	0.49	0.04	

**, Significant at 0.01 probability level.

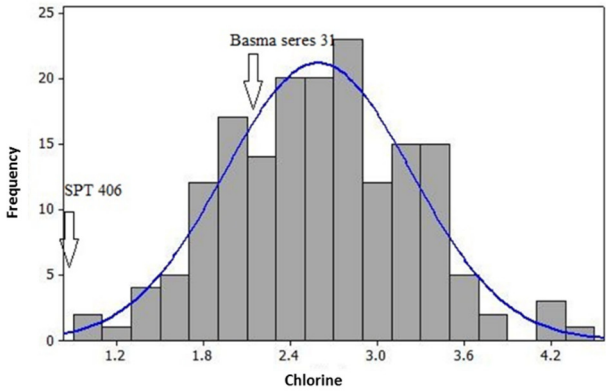

Figure 1. Frequency distribution of chloride accumulation in F2:3 families and parental lines of oriental tobacco.

Genetic linkage map

In this study, 162 SSR and 80 ISSR markers were used to construct the genetic linkage map in oriental tobacco. Twenty- three SSR (14.2%) and 29 ISSR (36.25%) markers were polymorphic between the parents. Low level of genetic diversity has been reported in many studies [25; 31; 40; 43]. The existence of low genetic diversity within cultivated oriental tobacco has been attributed to the narrow genetic background and self-pollination behaviour of plant [25]. Deviation of genotypic frequency of the polymorphic markers from Mendelian inheritance was assessed using Chi-square test. The result showed that there was no deviation from Mendelian segregation ratio. The Mendelian inheritance of molecular markers provides a genetic framework for the dissection of polygenic traits [24] and can pave the way for the identification of candidate loci controlling the inheritance of complex traits [38]. Thirty-four out of 52 polymorphic markers were mapped onto 7 linkage groups with a total length of 570.8 cM. Eighteen polymorphic markers could not be mapped and eliminated from the mapping try. The number of markers in each linkage group varied from 2 to 12 (Figure 2). Distance between two mapped markers was 17.3 cM. Recently, two highly saturated genetic linkage map of tobacco were constructed on 196 backcross individuals using 4138 and 2162 single nucleotide polymorphism (SNP) markers with a total length of 1944.74 and 2000.9 cM based on reference and without reference genome, respectively. The markers were mapped to 24 linkage groups [38]. Bindler et al. [4] mapped 2318 SSR markers to 24 linkage groups covering 3270 cM of the tobacco genome. In this study, three linkage groups were in common with the linkage groups constructed by Bindler et al. [4].

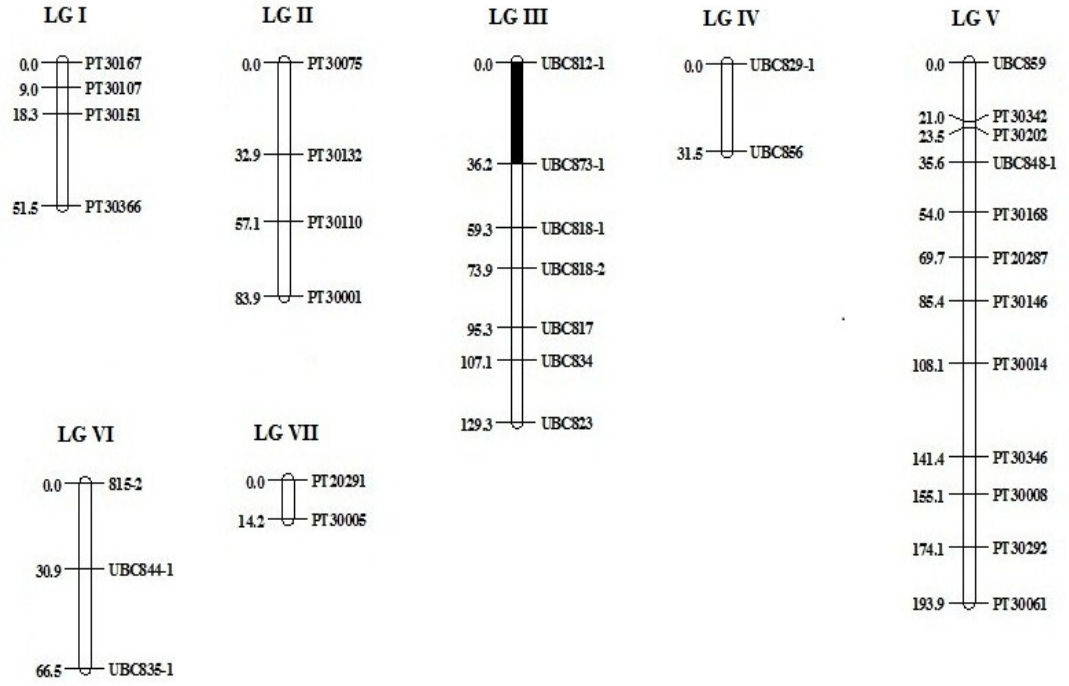

Figure 2. Linkage map of SSR and ISSR markers in $F_{2:3}$ population of oriental tobacco derived from the cross between 'Basma Seres 31' × 'SPT 406'. Black region on LG III represents intervals associated with identified QTL.

Table 2. QTL affecting chloride accumulation in the $F_{2:3}$ families derived from a cross between 'Basma Seres 31' and 'SPT 406' tobacco lines.

Method	QTL	LG	Position [a]	LOD [b]	R^2 [c]	a [d]	d [e]	d/|a| [f]
CIM	Chl_{CIM}	3	0.0	1.41	0.127	0.16	0.57	3.56

[a]Position of the QTL from the top of linkage group (LG), [b]Log10 likelihood ratio (likelihood that the effect occurs by linkage/likelihood that the effect occurs by chance), [c]Percentage of phenotypic variance explained by identified QTL, [d]Additive gene effect of putative QTL, [e]Dominance gene effect of putative QTL, [f]degree of dominance.

QTL analysis

To identify QTLs controlling chloride accumulation in leaves, CIM was used. One QTL on linkage group 3 was identified for trait, which explains 12.7% of the phenotypic variation (Table 2, Figure 2). In this study, the additive (16%) value of QTL was less than dominance value (57%). Similar result was reported by Hatami Maleki et al. [22]. In compliance with the present result, diallel analysis revealed that the importance of generalcombining ability (GCA; due to additive gene action) was less than specific combining ability (SCA; due to dominance gene action) in controlling the Cl^- accumulation [12]. Hence, the development of hybrid could be recommended to achieve genetic improvement for low Cl^- accumulation. However, complementary studies need to be performed before final decision. The positive effects of identified QTL indicate that responsible allele for chloride accumulation has been transferred from paternal line (SPT 406) to progenies. Mapping of QTLs linked to different traits have been reported in Nicotiana species. A genetic map was constructed using a doubled haploid population of flue-cured tobacco based on 169 markers including 11 ISSR markers and 158 random amplified polymorphism DNA (RAPD) markers. The map consisted of 27 linkage groups and spanned 2094.6 cM of genome with an average marker distance of 14.8 cM [37]. A genetic linkage map was constructed using 99 F2 population from the cross *Nicotiana plumbaginifolia* × *Nicotiana longiflora* based on 69 RFLP and 102 RAPD loci. The map consisted of nine major linkage groups, each containing more than nine marker loci, and spans 1062 cM of tobacco genome [29]. Agacka-Mołdoch et al. [1] determined four genomic regions on four different linkage groups that associated with four germination-related traits in 122 recombinant inbred lines. Four QTLs associated with easy curing

potential were detected using a F2 mapping population and 75 SSR markers [33]. Julio et al. [25] identified one to three QTLs for agronomic and chemical traits in tobacco. Narrow genetic studies in tobacco could be probably due to the difficulty of detecting DNA polymorphisms within N. tabacum [25].

The DNA marker that is linked to chloride accumulation may be used as molecular tools for MAS in plant breeding. The successful application of MAS relies on the tight association between the marker and QTL responsible for the trait. The new genomic tools such as next generation sequencing (NGS) accelerate the identification of markers tightly linked to target genomic regions. Although the constructed map has only contained 7 linkage groups and limited markers, this map can be further used as frame map for saturating by new markers.

REFERENCES

[1] Agacka-Mołdoch, M., Nagel, M., Doroszewska, T., Lewis, R. S. and Börner, A. 2015. Mapping quantitative trait loci determining seed longevity in tobacco (*Nicotiana tabacum* L.). Euphytica, 202, 479-486.

[2] Arumuganathan, K. and Earle, E. D. 1991. Estimation of Nuclear DNA Content of Plants by Flow Cytometry. Plant Molecular Biology Reporter, 9(3), 229-233.

[3] Basten, C. J., Weir, B. S. and Zeng, Z. B. 2003. QTL Cartographer: A Reference Manual and Tutorial for QTL Mapping. USA: Department of Statistics, North Carolina State University.

[4] Bindler, G., Plieske, J., Bakaher, N., Gunduz, I., Ivanov, N., Van der Hoeven, R., Ganal, M., and Donini, P. 2011. A high-density genetic map of tobacco (*Nicotiana tabacum* L.) obtained from large scale microsatellite marker development. Theoretical and Applied Genetics, 123, 219-230.

[5] Bozhinova, R. 2012. Investigation of chloride concentration in burley tobacco varieties. Tutun/Tobacco, 62 (7-12), 103-108.

[6] Brandsma, M., Wang, X., Diao, H., Kohalmi, S. E., Jevnikar, A. M., and Ma, S. 2009. A proficient approach to the production of therapeutic glucagon-like peptide-1 (GLP-1) in transgenic plants. The Open Biotechnology Journal, 3, 57-66.

[7] Burtin, D., Chabre, H., Olagnier, D., Didierlaurent, A., Couret, M. N., Comeau, D., Wambre, E., Laparra, H., Van Overtvelt, L., Montandon, F., Batard, T., Jonval, V., Lorphelin, A., Merle, C., Berrouet, C., Parry, L., Gomord, V., Van Ree, R. and Moingeon, P. 2009. Production of native and modified recombinant Der p 1 molecules in tobacco plants. Clinical and Experimental Allergy, 39(5), 760-770.

[8] Chai, C. C., Chai L. G., Cai C. C., Lin G. P., Wang Y. and Xu F. S. 2009. Construction of genetic linkage map of burley tobacco (*Nicotiana tabacum* L.) and genetic dissection of partial traits. Acta Agronomica Sinica, 35(9), 1646-1654.

[9] Chari, M. S. 1995. Role of research in the improvement of productivity and quality of Indian flue cured Virginia tobacco. Central Tobacco Research Institute: Rajahmundry.

[10] ChaoQiang, J., DeCheng, L., HuoYan, W., DongQi, Z., Jia, S., YiFeng, Y., ChuanJie, S. and ChaoLong, Z. 2016. Variance analysis on potassium and chloride contents of flue-cured tobacco among different varieties and producing areas in Bozhou. Journal of Agricultural Science and Technology, 18(1), 120-128.

[11] Churchill, G.A. and Doerge, R.W. 1994. Empirical threshold values for quantitative trait mapping. Genetics, 138, 963-971.

[12] Darvishzadeh, R. and Alavi, R. 2011. Genetic analysis of chloride concentration in oriental tobacco genotypes. Journal of Plant Nutrition, 34(7), 1070-1078.

[13] Darvishzadeh, R., Alavi, R. and Sarafi, R. A. 2011. Genetic variability for chloride concentration in oriental tobacco genotypes. Archives of Agronomy and Soil Science, 57(2), 167-177.

[14] Johnson, S. C. 1999. Tobacco: Production, Chemistry and Technology. (1th ed). Oxford and Malden (Massachusetts): Blackwell Science,

[15] Dellaporta, S. L., Wood, J. and Hicks, J. B. 1983. A plant DNA minipreparation: version II. Plant Molecular Biology Reporter, 1(4), 19-21.

[16] Ek, M., Eklund, R. and Venpost, R. 2005. Microsatellite markers for powdery mildew resistance in pea (*Pisum sativum* L.). Hereditas, 142, 86-91.

[17] Fulton, T. M., Van der Hoeven, R., Eannetta, N. T. and Tanksley, S. D. 2002. Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell, 14, 1457-1467.

[18] Garner, W. W. 1930. Role of chloride in nutrition and growth of the tobacco plant and its effect on the quality of the cured leaf. Journal of Agricultural Research, 40(7), 627-648.

[19] Ganapathi, T. R., Suprasanna, P., Rao, P. S. and Bapat, V. A. 2004. Tobacco (*Nicotiana tabacum* L.) a model system for tissue culture interventions and genetic engineering. Indian Journal of Biotechnology, 3(2), 171-184.

[20] Givry, S. D., Bouchez, M., Chabrier, P., Milan, D. and Schiex, T. 2005. CARHTA GENE: multi population integrated genetic and radiation hybrid mapping. Bioinformatics, 21(8), 1703-1704.

[21] Guler Gumus, S. 2008. Economic analysis of oriental tobacco in Turkey. Bulgarian Journal of Agricultural Science, 14, 470-475.

[22] Hatami Maleki, H., Karimzadeh, G., Darvishzadeh, R., Naghavi, M. R. and Sarrafi, A. 2013. Identification of QTLs associated with low chloride accumulation in oriental tobacco. Genetika, 45(3), 855-864.

[23] Ishizaki, H. and Akiya, T. 1978. Effects of chloride on growth and quality of Tobacco. Japan Agricultural Research Quarterly, 12(1), 1-6.

[24] Jansen, R. C. 1996. Complex plant traits: time for polygenic analysis. Trends in Plant Science, 1: 89-94.

[25] Julio, E., Denoyes-Rothan, B., Verrier, J. L. and Dorlhac de borne, F. 2006. Detection of QTLs linked to leaf and smoke properties in Nicotiana tabacum based on a study of 114 recombinant inbred lines. Molecular Breeding, 18(1), 69-91.

[26] Kenton, A., Parokonny, A. S., Gleba, Y. Y. and Bennett, M. D. 1993. Characterization of the *Nicotiana tabacum* L. genome by molecular cytogenetics. Molecular and General Genetic, 240, 159-169.

[27] Leitch, I. J., Hanson, L., Lim, K. Y., Kovarik, A., Chase, M. W., Clarkson, J. J. and Leitch, A. R. 2008. The ups and downs of genome size evolution in polyploid species of Nicotiana (Solanaceae). Annals of Botany, 101(6), 805-814.

[28] Lewis, R. S. 2011. In: Kole C, ed. Wild crop relatives: genomic and breeding resources, plantation and ornamental crops. Berlin: Springer-Verlag Berlin Heidelberg.

[29] Lin, T. Y., Kao, Y. Y., Lin, S., Lin, R. F., Chen, C. M., Huang, C. H., Wang, C. K., Lin, Y. Z. and Chen, C. C. 2001. A genetic linkage map of *Nicotiana plumbaginifolia* / *Nicotiana longiflora* based on RFLP and RAPD markers. Theoretical and Applied Genetics, 103, 905-911.

[30] Prasad, R. 2006. Textbook of Field Crops Production. New Delhi: Indian Council of Agricultural Research.

[31] Ren, N. and Timko, M. P. 2001. AFLP analysis of genetic polymorphism and evolutionary relationships among cultivated and wild Nicotiana species. Genome, 44(4), 559-571.

[32] Rieseberg, L. H., Archer, M. A. and Wayne, R. K. 1999. Transgressive segregation, adaptation and speciation. Heredity, 83, 363-372.

[33] Tan, X., Xu, X., Wang, N., Zhang, X., Ren, J., Xiao, B., Xu, J., Wang, W., Wang, C., Hao, X. and Zhang, Z. 2012. QTLs related to the easy curing potential mapped in flue-cured tobacco. Plant Gene and Trait, 3(6), 28-33.

[34] Tong Z., Jiao T. and Wang F. 2012. Mapping of quantitative trait loci conferring resistance to brown spot in flue-cured tobacco (*Nicotiana tabacum* L.). Plant Breeding, 131(2), 335-339.

[35] Vontimitta V. and Lewis R. S. 2012. Mapping of quantitative trait loci affecting resistance to *Phytophthora nicotianae* in tobacco (*Nicotiana tabacum* L.) line Beinhart-1000. Molecular Breeding, 29(1), 89-98.

[36] Wu F., Mueller L. A., Crouzillat D., Pétiard V. and Tanksley S. D. 2006. Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: a test case in the euasterid plant clade. Genetics, 174, 1407-1420.

[37] Xiao, B. G., Xu, Z. L., Chen, X. J., Shen, A. R., Li, Y. P. and Zhu, J. 2006. Genetic linkage map constructed by using a DH population for the flue-cured tobacco. Acta Tabacaria Sinica, 12, 35-40.

[38] Xiao, B. G., Tan, Y., Long, N., Chen, X., Tong, Z., Dong, Y. and Li, Y. 2015. SNP‐based genetic linkage map of tobacco (*Nicotiana tabacum* L.) using next‐generation RAD sequencing. Journal of Biological Research, 22,11.

[39] Xu, Y. and Crouch, J. H. 2008. Marker-assisted selection in plant breeding: from publications to practice. Crop Science, 48(2), 391-407.

[40] Yang, B. C., Xiao, B. G., Chen, X. J. and Shi, C. H. 2007. Assessing the genetic diversity of tobacco germplasm using inter simple sequence repeat and inter-retrotransposon amplification polymorphism markers. Annals Applied Biology, 150(3), 393-401.

[41] Zeng, Z. B. 1993. Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proceedings of the National Academy of Sciences, 90(23), 10972-10976.

[42] Zeng, Z. B. 1994. Precision mapping of quantitative trait loci. Genetics, 136 (4), 1457-1468.

[43] Zhang, H. Y., Liu, X. Z., Li, T. S. and Yang, Y. M. 2006. Genetic diversity among flue-cured tobacco (*Nicotiana tabacum* L.) revealed by amplified fragment length polymorphism. Botanical Studies, 47, 223-229.

[44] Zimmerman, J. L. and Goldberg, R. B. 1977. DNA sequence organization in the genome of *Nicotiana tabacum*. Chromosom, 59(3), 227-252.

نقشه‌یابی QTLهای مرتبط با تجمع کلر در برگ توتون شرقی (*Nicotiana tabacum* L.) با استفاده از جمعیت $F_{2:3}$ تلاقی Basma Seres 31 × SPT 406

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چکیده

کلرید به عنوان مهم‌ترین ریز مغذی در تولید توتون در نظر گرفته می‌شود. اما مقدار زیاد تجمع کلرید در برگ‌های توتون اثرات سوء بسیاری بر کیفیت توتون مانند ظرفیت سوزش (Burning capacity) دارد. شناسایی مکان‌های صفت کمّی (QTL) که در تجمع کلرید نقش دارند، برای بهبود کیفیت توتون مفید است. هدف از این مطالعه شناسایی مناطق ژنومی مرتبط با تجمع کلرید با استفاده از نقشه‌یابی جمعیت شامل ۲۲۵ خانواده $F_{2:3}$ حاصل از تلاقی لاین‌های "Basma Seres 31" و "SPT 406" می‌باشد. نقشه پیوستگی با ۲۳ نشانگر ریز ماهواره (SSR) و ۲۹ نشانگر نواحی بین توالی‌های تکراری ساده (ISSR) چند شکل ساخته شد که ۵۷۰/۸ سانتی‌مورگان از ژنوم توتون را پوشش می‌داد. سی و چهار عدد از این نشانگرهای چند شکل در ۷ گروه پیوستگی قرار گرفتند. فاصله بین دو نشانگر مجاور ۱۷/۳ سانتی‌مورگان بود. برای شناسایی QTLهای کنترل‌کننده تجمع کلرید از نقشه‌یابی فاصله‌ای مرکب (CIM) استفاده شد. یک QTL برای تجمع کلرید در گروه پیوستگی ۳ شناسایی شد. درصد واریانس فنوتیپی (R^2) توجیه شده توسط این QTL7/12 درصد بود. ارتباط معنی‌داری بین نشانگرهای ISSR و تجمع کلرید یافت نشد. نتیجه تلاش حاضر می‌تواند پایه‌ای برای انتخاب به کمک نشانگر (MAS) در برنامه‌های اصلاحی توتون باشد.

کلمات کلیدی: تجمع کلرید، نقشه‌یابی فاصله‌ای مرکب، نقشه‌یابی پیوستگی، توتون شرقی