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Insilico Identification of Markers for Determinacy in Tomato

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Abstract

Tomato is a major vegetable crop that has achieved tremendous popularity over the last century. The market value for the tomato is highly fluctuating because of the season bound yield of the crop and the usage of determinate cultivars. The determinate type of tomatoes is preferred by the farmers for outdoor cultivation because of less maintenance, short duration and easy harvest. However indeterminate tomato cultivars are suitable for greenhouse cultivation and preferred by the food processing industries. Identification of markers for the growth habit determination will be useful to improve the tomato cultivars based on the preference of farmers and consumers. Available Single Nucleotide Polymorphism (SNP) data of six tomato cultivars of both determinate and indeterminate types were compared in the present study. The genome wide SNPs were analysed for SNPs differentiating the determinate and indeterminate nature of the crop. From a list of 1,473,798 SNPs, 986 SNPs were found to be differentiated the varieties compared. The locations of the obtained SNPs were identified and 254 SNPs were present within the transcribed region and the rest were found within the intergenic region. These SNPs were considered for further analysis, with respect to growth habit regulation. From the list of SNPs present in transcribed region, SNPs associated with the genes regulating the growth habit were identified. The SNPs within the intergenic region were analysed for their association with the non-coding RNAs. The results showed that 58 SNPs spread over 15 genes were found to be involved in regulation of growth habit. These SNPs could be validated using determinate and indeterminate cultivars and could be utilized for marker assisted breeding programs favouring growth habit determination.

Key words: Tomato, Growth type, Marker gene, SNP, determinate, indeterminate flowering

Introduction

Solanaceaous crops have been subjected to intensive human selection. The phylogenetic classification of the *Solanaceae* has been revised recently (Fernando *et al*. 2012). Tomato is a major Solanaceous crop, having wide range of application in food processing industries. Hybrids and open pollinated tomato cultivars used for sauce / ketchup production usually have determinate growth habit and are preferred by the farmers for cultivation because of their short duration, easy maintenance and harvest. However most of the cultivars used as salads are having indeterminate growth habit and suitable for greenhouse cultivation (Pnueli *et al.* 1998). Industries prefer the indeterminate verities because of the flavour and growth habit but it is generally not of farmer's choice. Crop cultivation under greenhouse/ polyhouse condition ensures reduced pest and disease infestation and controlled environmental conditions like temperature, humidity result in increased yield (Evans *et al.* 2010). Hence the impetus could be on cultivars/ hybrids with indeterminate growth habits. Though determinate types are easy for cultivation, indeterminate types can ensure higher yields under green house cultivation and can control the fluctuation on market value of tomato (Amina *et al.* 2012). These

determinate and indeterminate growth habits are controlled by genes regulating the growth. It was found out that the *SP* (Self pruning) gene is strongly associated with growth habit in tomato cultivars due to the presence of a SNP (T227C) in the *SP* gene (Pnueli *et al*, 1998). Single nucleotide polymorphisms and indels are various sources of polymorphic markers for use in the high-resolution genetic mapping of traits (Rafalski 2002). Exploration of SNPs controlling the growth habit is essential to differentiate the cultivars. Insilico methods would efficiently find out the SNPs of our interest.

The present study focuses on insilico identification of the SNPs present in the genes that are involved in controlling the growth habit of tomato. Identified SNPs can be verified and used as markers for the growth habit (determinate and indeterminate) determination. Besides, these findings can also be used to improve the tomato cultivars/land races based on the preference of farmers as well as consumers/ industry. Shirasawa *et al.,* 2013 have published the details of whole genome SNP for six different cultivated tomatoes in KaTomicsDB: Kazusa Tomato Genomics Database **(**Shirasawa *et al.* 2013). These genome wide SNP data was utilized to identify the SNPs differentiating determinate and indeterminate growth types in tomato.

Materials and methods

Collection of SNP data

The genome wide SNP data of six different tomato cultivars namely Furicoma, M82, Regina, Ailsa Craig, Ponderosa, Tomato chuukanbohon nou 11 and the reference cultivar HEINZ 1706 were available in the Kazusa Tomato Genomic Database. The same was obtained from Kenta Shirasawa, Kazusa DNA Research Institute, Japan as a single file.

Comparison of SNPs between the determinate and indeterminate cultivars

The above six cultivars were grouped into determinate and indeterminate types based on their respective phenotype from the literature and the SNP variation in the *SP* gene. All the other SNPs available in the dataset were grouped against the two main traits of determinate and indeterminate growth habits. The SNPs differentiating the determinate and indeterminate type were manually sorted and analysed further.

Categorization of SNPs

The genomic location of the above sorted SNPs were identified from the database and characterized based on their location as coding region, UTR, intronic region and intergenic region (Additional file 1). The SNPs present in the transcribed region (coding region, UTR and intronic region) were taken for further analysis. The respective genes for the selected SNPs were listed (Additional file 1). The SNPs located in intergenic region were analysed separately for their function towards growth type determination in tomato.

Analysis of genic SNPs for possible growth regulatory role

SNPs located within genes were analysed for their role in regulation of growth habit in Solanaceous plants particularly tomato through literature and microarray data search.

Transition of vegetative to flowering stage as well as flowering to vegetative stage could be directly correlated to growth habit regulation (determinate/ indeterminate). So the gene expression profile of differentially expressed genes between terminating flower (*tmf)* mutant and wild type (M82) tomato (MacAlister *et al.* 2012) were analysed. And also Arabidopsis transcription profile at transition of vegetative to reproductive stage (Wellmer *et al.* 2006) was utilized to select the candidate genes regulating the growth habit and the respective SNPs. The SNPs present in the genes coding for unknown protein were predicted functionally using nucleotide blast search against the SNP containing genic region (Additional file 2). The predicted genes were analysed further for their role in regulation of growth habit.

Analysis of SNPs in the intergenic region

The SNPs present in intergenic region were compared against non coding regulatory RNAs of tomato, such as micro RNAs (miRNAs) from miRBase database. The miRNAs of the related crops (Arabidopsis, Potato, and Tobacco) were also searched against tomato genome (nucleotide blast) and the predicted genomic regions also analysed for the presence of SNPs.

Results

SNPs differentiating growth habit

The six tomato cultivars for which SNP data was available were differentiated as determinate and indeterminate types. The difference could be correlated from their phenotypes (Marti E *et al.* 2006, Carmel-Goren L *et al.* 2003, Eshed Y *et al.* 1995) and confirmed by the SNP mutation in *SP* gene. Cultivars, Furicoma, M82 and Regina were designated as determinate, cultivars Ailsa Craig, Ponderosa and Tomato chuukanbohonnou 11 were the indeterminate tomato cultivars. The total number of genome wide SNPs observed in these six tomato cultivars amounted to 1,473,798. Among them about 986 SNPs were found to be demarcating the determinate and indeterminate type of tomato cultivars (Additional file 1).

Category of SNPs

The above observed 986 SNPs were categorized based on their location as genic (coding region, UTR and intronic region) and intergenic SNPs (Table 1).

Table 1: SNP categories based on the location

SNP location	No of SNPs	Total SNPs
Coding region	44	Transcribed region- 254
UTR	17	
Intronic region	193	
Intergenic region	732	Untranscribed region- 732

Among the 254 SNPs in the transcribed region 205 SNPs were present in genic regions of known proteins and remaining 49 were found to be present in coding region of 21 protein of unknown function.

Association of genic SNPs to growth habit regulatory genes

Among the SNPs present in the transcribed region (genic) 56 SNPs were found to be present in 14 genes regulating growth habit. This is identified from transcription profiles and available literatures (Table 2). Functions of the unknown protein coding genes carrying SNP were predicted insilico (Additional file 2). The analysis resulted a single gene associated to growth habit regulation that carries two SNPs within (Table 3).

Table 2: SNPs present in known growth habit regulatory genes

Sl. No.	**SNP NAME**	**SNP TYPE**	**GENE TAG**	**FUNCTION**
1	SL2.40ch06_42362163Y	Missense	Self- pruning	Plant growth habit
2	SL2.40ch05_60057668S	Missense	BAHD family acyltransferase	Plant growth habit
3	SL2.40ch01_74591789S	Intron	MADS-box transcription factor	Plant growth habit and flower development
4	SL2.40ch09_986685Y	Intron	Calmodulin-binding protein	Flower development
5	SL2.40ch09_986700R	Intron		
6	SL2.40ch09_987809K	Intron		
7	SL2.40ch09_985322M	UTR		
8	SL2.40ch09_985364Y	UTR		
9	SL2.40ch02_28536957M	Missense	Heat shock protein (*HSP*)	Plant growth habit
10	SL2.40ch02_28535719Y	Intron		
11	SL2.40ch02_28537544K	Intron		
12	SL2.40ch02_31762917R	Synonymous	RNA binding protein (glycine rich protein)	Plant growth habit
13	SL2.40ch02_31763079R	Intron		
14	SL2.40ch02_31763218W	Intron		
15	SL2.40ch02_31763676S	Intron		

16	SL2.40ch09_1164029W	Missense	MYB transcription factor	Plant architecture
17	SL2.40ch09_1163799R	Intron		
18	SL2.40ch09_57025S	Missense	LRR receptor-like protein kinase	Plant and inflorescence architecture
19	SL2.40ch05_60381256K	Missense	Glycogen synthase kinase (*GSK)*	Flower development
20	SL2.40ch05_60381258M	Synonymous		
21	SL2.40ch05_60383011Y	intron		
22	SL2.40ch05_60095288Y	Missense	Serine carboxy peptidase 1	Plant growth habit and flower development
23	SL2.40ch05_60070078Y	Intron		
24	SL2.40ch05_60071054S	Intron		
25	SL2.40ch05_60072561Y	Intron		
26	SL2.40ch05_60075274Y	Intron		
27	SL2.40ch05_60075434R	Intron		
28	SL2.40ch05_60095572K	Intron		
29	SL2.40ch05_60097198R	Intron		
30	SL2.40ch05_60102301Y	Intron		
31	SL2.40ch05_60102667M	Intron		
32	SL2.40ch09_2925238Y	Missense	Hydrolase α/β fold family protein	Plant architecture and branching
33	SL2.40ch09_975378R	Missense		
34	SL2.40ch09_976611M	Synonymous		
35	SL2.40ch09_2924952R	UTR		
36	SL2.40ch09_2927031R	Intron		
37	SL2.40ch09_2930814S	Intron		
38	SL2.40ch09_2931920Y	Intron		
39	SL2.40ch09_2951234Y	Intron		
40	SL2.40ch09_2962242W	Intron		

41	SL2.40ch09_973942K	Intron		
42	SL2.40ch09_973992R	Intron		
43	SL2.40ch09_974080R	Intron		
44	SL2.40ch09_974111W	Intron		
45	SL2.40ch09_974173Y	Intron		
46	SL2.40ch09_974321S	Intron		
47	SL2.40ch09_974403Y	Intron		
48	SL2.40ch09_974418S	Intron		
49	SL2.40ch09_975082R	Intron		
50	SL2.40ch09_975192R	Intron		
51	SL2.40ch09_937159R	Synonymous	PWWP domain-containing protein	Flower development
52	SL2.40ch03_58641921K	Missense	WD repeat containing protein	Plant growth and flower development
53	SL2.40ch02_31792871W	Missense	UDP-glycosyltransferase	Plant architecture
54	SL2.40ch03_58239956Y	UTR		
55	SL2.40ch02_31792192Y	Intron		
56	SL2.40ch09_1516769R	Intron		

Table 3: SNPs present in growth habit regulating genes (Predicted)

SL. NO.	SNP NAME	SNP TYPE	GENE TAG PREDICTED	FUNCTION
1	SL2.40ch11_3679198K	Missense	MADS-box transcription factor JOINTLESS gene	Plant growth habit and flowering
2	SL2.40ch11_3724494R	Intron		

These SNPs are to be validated involving tomato germplasm to identify markers able to discriminate determinate/indeterminate growing tomato.

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Association of intergenic SNPs to growth habit regulatory genes

The intergenic SNPs were analysed for their presence in known miRNAs and predicted miRNA regions of the tomato. There were no SNPs present in those regions. Novel miRNAs identified in future may carry significance to these intergenic regions harbouring SNPs.

Discussion

SNP represent a difference in a single nucleotide and has helped as biological markers locating genes that are associated with trait. The current study identifies SNP mutation found in 15 candidate genes (Table 2 & 3) including self- pruning *(SP)* gene with an ability to determine the growth habit of tomato. These mutations alter the function or expression of the candidate genes, which are involved in growth habit regulation in tomato.

Results of our present study show that the mutation (T227C) in the *SP* gene located in the sixth chromosome of tomato leads to an amino acid change (L76P) in the protein. This SNP mutation has been exploited towards growth habit selection in tomato (Pnueli *et al.* 1998, Jones *et al.* 2007). Lifschitz *et al.* in 2006 described that the single flower truss (*SFT*) a regulator of flowering-time and shoot architecture encodes the tomato orthologue of flowering locus T *(FT*). This is a major flowering integrator gene in *Arabidopsis*. Systemic signals initiated by *SFT* interact with the *SP* gene to regulate, vegetative to reproductive transitions in the background of two flowering systems, for primary apices and sympodial shoots.

A missense mutation in BAHD acyltransferase identified in the present study is found to distinguish the cultivars with respect to the growth habit. A previous study states that the overexpression of a BAHD family of acyltransferases, ABS1/ At4g15400, has been identified as the cause to induce dwarf phenotypes in Arabodopsis (Wang *et al.* 2012). The observed mutation in this particular gene may thus be involved in determining plant architechture and hence impose a meaningful influence towards growth type modification between the determinate and indeterminate cultivars.

Our study also reveals that there is an intronic SNP mutation of the MADS box transcription factor. This type of mutation generally leads to alternate splicing, that could alter the expression profile resulting in altered phenotypes. Melzer *et al.* (2008) have discussed that the MADS box proteins, suppressor of overexpression of constans 1 (*SOC1*) and Fruitfull (*FUL*) not only control flowering time, but also affect determinacy of all meristems in *Arabidopsis*. In addition, down-regulation of both the above proteins established phenotypes common to the growth types of *Solanaceous* plants, suggesting their involvement in the prevention of secondary growth and longevity in annual plant types. In addition, Immink *et al.* (2012) found out that several MADS domain proteins, including *SOC1* heterodimers, are able to bind to *SOC1* regulatory sequences. In turn, the encoded floral homeotic MADS domain proteins appear to bind *SOC1* regulatory sequences. Thus, a series of effects with regards to flowering time and determinacy traits could be potentially altered as an effect of this SNP mutation.

This analysis has highlighted 3 SNPs in intronic regions and 2 SNPs in the UTR of the gene coding for Calmodulin-binding protein. This protein has significance with respect to negative regulation of flowering in tobacco. Hua *et al.* (2004) have concluded that the transgenic tobacco plants overexpressing *NtCBK1*- a Calmodulin-binding protein, display a late-flowering phenotype, establishing that *NtCBK1* could function as a negative regulator of flowering.

The heat shock protein (HSP) gene, one of the above listed 12 genes, posses 3 identified SNPs from our comparison. HSP is well established to influence growth determination. Saad *et al.* in 2008 have reported that heat shock protein regulate the growth and development of tomato both under greenhouse and field condition. In this the transgenic tomato plants constitutively expressed a HSP reported with a change in the architecture of flower clusters (simple to dichotymously branched cyme).

Considering the intronic regions 3 SNPs and single synonymous mutation in RNA binding protein located in chromosome 2 aided in differentiating the determinate and indeterminate cultivars. Lim *et al.*, (2004) observed an *Arabidopsis* gene flowering locus K (*FLK)*, and shown to encode a putative RNA binding protein with K homology motifs. FLK regulates the autonomous flowering pathway via Flowering Locus C (FLC). It is evident that different RNA binding proteins are involved in the posttranscriptional regulation of flowering time in *Arabidopsis*.

Two SNPs (A missence and an intronic SNP) have been identified which may contribute to regulate the gene towards the growth habit determination. However, their roles in regulating plant growth and development remain largely unknown. Liu *et al.* (2013) showed that PtrMYB192 function as a transcription activator. Transgenic *Arabidopsis* plants overexpressing PtrMYB192 showed delayed flowering time under both long day and short day conditions, indicating that PtrMYB192 regulates flowering. Hence our results are in conjunction towards the identified SNPs being able to delineate the determinate and indeterminate tomato cultivars.

GSK3 (glycogen synthase kinase 3) gene is located in chromosome 5 of tomato. This gene carries 3 SNPs (A missence, a synonymous and an intronic SNP) differentiating the growth habit. Homologs of two *Arabidopsis* GSK3 genes with genetically confirmed roles in floral development, AtSK11 and AtSK12, exhibit floral preferential expression in *Arabidopsis* suggesting evolutionary conservation of their floral functions (Qi *et al.* 2013). Thus GSK 3 could influence growth type determination in tomato also.

Our study has picked 9 intronic and one missense mutation in Serine carboxy peptidase 1 gene. Brassinosteroid-insensitive 1 (*BRI1*) is a Serine carboxy peptidase 1 of *Arabidopsis*, that encodes a cell surface receptor for brassinosteroids. Li *et al*., (2001) reported that the serine carboxypeptidase encoded by *BRS1* is involved in the BRI1 signaling pathway. Mutations in BRI1 severely affect plant growth and development. So the above mutations may result in differential expression of this gene and thereby control growth habit.

Hydrolase alpha/beta family protein was identified with 19 SNPs in our study. An earlier study reported that, DAD2 is an alpha/beta hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone (Hamiaux *et al.* 2012), there by the mutation in this gene can regulate and have influence on the plant architecture of tomato.

In the present study there is a synonymous SNP mutation observed in PWWP domain containing protein. The *Arabidopsis* genome carries at least 19 PWWP domain proteins. The PWWP domain is conserved among eukaryotes. It was first portrayed as a nonspecific DNA binding motif. The PWWP domain containing proteins were shown to harbor chromatin targeting ability in most of the eukaryotes (Ge *et al.* 2004; Wang *et al.* 2009; Vezzoli *et al.* 2010). The ability of the PWWP domain to classify the tomato cultivars based on growth habits makes it an interesting candidate to be explored upon.

Nearly half of all trait-associated genomic regions are reported as intergenic in genome wide association studies. While a few of these regions possibly will **function** solely as DNA elements, it is **now known that intergenic regions can be transcribed**, and a **growing list of functional noncoding RNA genes within intergenic regions has** become known**.** The SNPs in the non coding sequence also play important role by regulating the genes controlling the growth habit. In this present study the SNPs present in the intergenic regions were listed and analysed for their presence in available list of miRNAs but the presence of SNPs were not observed in that region. Further exploration of novel miRNAs may result in significant findings relevant to the growth habit of tomato. Correlations of the growth habit determining genes were done effectively from the SNPs available in the coding regions reported. However, it is to be noted that majority of the SNPs were located in the intergenic regions.

All the above SNPs analysed are promising DNA features that could efficiently differentiate a determinate from an indeterminate plant type in tomato. DNA analysis using tomato cultivars/ germplasm could essentially lead to genotypic identification of cultivars for growth habit. Thus validation of the above identified SNPs could result in development of markers that can differentiate tomato genotypes for growth habit.

Conclusion

Identifying the growth habit of the crops is important to select a proper variety with a specific purpose. These can serve a better option for the improvement of crops by the breeders as well as farmers. The different genes discussed above could exhibit varying regulatory functions with respect to growth habit determination, flowering control and plant and inflorescence architecture. Validation of the identified SNPs utilizing a reliable Germplasm can lead to an enhanced understanding of the same. The germplasms may differ in gene regulation due to the habitat and other environmental factors. The SNPs may be varied with geographical location and regulation of growth habit can be due to any of the above genes listed due to their varying regulatory control over the trait. Hence this finding of the SNPs and genes can a lead resource to screen the Germplasm and differentiate them for specific growth types. This could enhance the breeding process favouring development of particular growth characteristics in addition to other favourable traits based on farmer's demands and consumer

preferences. The findings can also be extended to other Solanaceous crops of economic importance.

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