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Full Length Research Paper

Complex traits mapping using introgression lines in pepper (*Capsicum annuum)*

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In this study we used introgression lines of *Capsicum annuum* to fine map the location of the traits in the chromosome responsible for fruit production, fruit development and fruit ripening. To identify specific regions of the genome for the measurable traits for production, development and ripening in pepper required further investigation using the introgression line approach. Forty-one introgression lines of pepper (*C. annum*) were used to map the QTLs for fruit production, fruit development and fruit ripening in chromosomes. The traits were evaluated in two glasshouses each with single and double culture cycles. The results confirmed our earlier results that the QTLs for fruit ripening were located at 75 - 100 cM in chromosome 4 and 0 - 100 cM in chromosome 8; QTLs for fruit production at 0 - 100 cM on chromosome 2; QTLs for fruit development at 0 - 50 cM on the chromosome 2 and 0 - 65 cM on chromosome 3. Genotype IL14 had a combination of small and large segments from chromosomes 7 and 10 suggesting that two QTLs were likely involved in the fruit ripening. Statistical analysis was done using genstat Software package. Significant differences (P ≤ 0.05) were found in in some of the lines for the traits tested.

Key words: *Capsicum annuum*, complex trait, mapping, chromosome, production, development, ripening, introgression lines.

INTRODUCTION

Capsicum annuum. L. (2n = 24) belongs to the family solanaceae. It is estimated that there are 23 (Scheper, 1997), 22 (Bosland and Zewdie, 2001 cited in Bosland, 1994) species of pepper. Progress in plant genome analysis made it possible to examine naturally occurring allelic variation underlying complex traits. Quantitative trait loci (QTL) analysis provides information relevant to agricultural traits by using molecular markers to identify regions of the genome affecting any measurable trait (Ahn and Hwang, 2003 cited McCough and Doerge, 1995 and Yano, 2001). Pepper is suited to the identification of QTLs across cultivars. The wild species of *Capsicum* represents a gene reservoir which can be used in breeding of commercial varieties. This helps in solving agricultural problems associated with pests, diseases and yield. The identification of QTL across species or cultivars using introgression lines (IL) is very useful. An extensive work to identify major genes that control fruit yield and quality parameters in *Capsicum* had been done. Two genes, Capsanthin Capsorubin Synthase and Phytoene Synthase are reported to influence mature fruit color changes, traits like plant height, fruit weight, fruit shape pericarp thickness and maturity (Paran,2001). Yield parameter is an important trait in pepper. Recent studies conducted by Chaim et al. (2003) on the presence of anthocyanin and Fs10.1 a major fruit - shape QTL in pepper concluded that the purple color of the foliage, flower and immature fruit of pepper is a direct effect of the accumulation of anthocyanin substances in the tissues. The research further suggests that these substances are controlled by an incomplete dominant gene located in chromosome 10 in *C. annuum* and *Capsicum chinense*. Previous research by Chaim et al., (2003) detected in the region Fs3.1 (Fs3.1 refers to the ratio of fruit length to fruit width) a major fruit shape quantitative trait locus in an intraspecific cross of *C. annuum* between the blocky and elongated - fruited inbreds and perennial. However, their results pointed out that the perenial allele at Fs3.1 increased fruit shape index, fruit elongation but reduces fruit width and pericarp thickness. Thus the research emphasized that the region Fs3.1 controls the shape and

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Abbreviations: QTL, Quantitative trait loci; **IL,** introgression lines; **ALFP,** amplified fragment length polymorphism; **BILs,** backcross inbred lines.

width development of the fruit. The shape and regularity of bell pepper (*C. annuum L.*) are determined at an early stage of flower development. Aloni et al. (1999) established that fruits formed on plants growing under night-temperatures at 18°C will drop. Their research noted that the removal of leaves from the lower part of the plant (source leaves) will reduce the effect of fruit removal on the shape of the flowers and on subsequent fruit morphology. The result of that study indicates that flower morphology in pepper is at least partly controlled by source - sink relationships. The research concluded that fruit removal helps assimilate which are normally transferred to developing fruit to be transported to the flower buds which subsequently swell. However a similar increase in assimilate translocation to flower buds may occur under low temperatures causing deformation of fruit.

Conventional mapping populations used in QTL analysis have several demerits in the accurate detection and fine mapping of traits. The major disadvantage includes low resolution power and the inability to identify QTL with small effects. Another problem in the conventional mapping population results from the interaction between two unlinked QTLs. To overcome these problems, an introgression line (IL) population which resembles the backcross inbred lines (BILs) was developed for the mapping of interspecific variation for quantitative traits in tomato (Ahn and Hwang, 2003 cited Eshed and Zamir, 1995). In this work the IL approach was used to precisely map the complex traits in the *Capsicum* genotypes we developed in the recent past. The decision to use IL approach stems from the many advantages it offers over the conventional and other approaches. In the past few years we had carried out several crossings with the help of classical plant breeding techniques and developed introgression Lines. The introgression lines were evaluated for QTLs for fruit production, fruit development speed and ripening. These traits are somewhere on the (donor line) pepper chromosomes. To the best of our knowledge only a limited work had been done on the subject in pepper and no precise mapping of the traits on the chromosome had been reported to date. We also tested our hypotheses based on results of Rao et al. (2003) and Ben-Cham et al. (2000) that traits for fruit development may be on chromosomes 2, 12, 7or10 and 11, the traits for fruit ripening were located in chromosomes 4, 8 and 9 and that the traits for higher production may be in chromosomes 11, 2 and 4 and our preliminary results in 2003(unpublished). In 2003 the QTLs for fruit development were found on chromosomes 2, 3, 4, 7 or10, 9, 12 on the (0-100, 0 - 65, 0 - 75, 35 - 40, 0-100, 0 - 67, 0 - 90) cM parts respectively. Traits for fruit ripening were found on chromosomes 2,4,7/10,8,9 on the (60 - 100,0 -75/75 -100, 35 - 40,0 -100,0 - 67)cM respectively and for the traits for production on chromosomes 2 and 3 on the (0 -100,0 - 65)cM respectively. The trial had its own control to ensure proper evaluation.

MATERIALS AND METHODS

Development of the introgression lines

The commercial father line used as a recurrent parent was crossed with a wild donor line to produce an F1 generation. The F1 progeny was backcrossed with the recurrent parent line to produce the BC1 generation. The father line was further used several times as recurrent parent to get a high level of elite lines. An exotic genome with a set of sixty introgression lines was developed. Using amplified fragment length polymorphism (ALFP) markers the ILs were selected from the genomic map. These lines were screened for multiple phenotypes and alleles of agricultural importance and plants that were identical to the recurrent line were selected and backcrossed, then selfed and again using markers a total of forty-one introgression lines with introgression segments at one or a part of a chromosome out of the lot were selected. The AFLP markers were used to locate the part of the DNA in the progeny from each parent and to select lines that contained only one part of the donor. The forty-one lines (shown in Tables 1 and 2) were used for trial in glasshouses P49 and P50. The glasshouse P49 had a year culture and the lines were used to evaluate for fruit production. Production can be best evaluated in a year culture so as to be able to compare with commercial practice. The other lines that were not required for production were put in glasshouse P50 that had two cultures per year.

Fruit production

In P49 ten introgression lines (ILs) in five replications were arranged in non-randomized block design of five rows. Five plants were placed on a 2 meter mat per block. The ILs and their chromosomal parts are shown in the result (Table 1). The distance between rows was 80 and 45 cm between plants. The plants were kept at day and night temperatures of 21 and 20°C respectively and fed with nutrient solution between 1.8 - 3.8 lm^{-2} in week 51 of 2005. However in week 2 of 2006, the plants were kept at 20°C day and 18°C night temperatures. The EC ranged between 3.5 - 4.0 and PH 6.6. RH varied from 54 - 90%. Border pepper plants were grown on both ends of the rows to enhance equal growing circumstances.

Fruit production refers to the number and weight of matured fruits collected per genotype. Collection of the fruits was done when they were 95 -100% ripe on Wednesdays of every week. Fruit weight in kg after harvest was taken using the Husky computer and a balance. The diameter in mm of the biggest fruit per genotype for each harvest was recorded.

Fruit development

In P50 a total of thirty-one lines were used for evaluation for fruit development and ripening. The lines (ILs and F1's of the ILs) with large and small chromosome segments were arranged in a complete randomized block design with 5 replicates per line (genotype) were placed in the glasshouse in November; 2005.The genotypes are shown in the result (Table 2). The plants were distributed in five rows. The distance between rows was 1m and between plants was 50 cm. Four plants were placed on a 2 m rock wool mat. The variety control refers to the hybrid obtained when the recurrent parent was crossed with the F1 generation. The other control is the recurrent parent. The temperature in the glasshouse was maintained at 23°C day and 21°C night from week 4 through week 21 and was slightly lowered at 22°C day and 19°C night from week 21 through week 28 to enhance fruit set. The relative humidity varied from 54% in week 4 to 100% in week 28.

Fruit developmental speed refers to the total number of days

Table 1. ILs chromosomes and parts and mean value for fruit production.

Genotype	Chr.#	cM	Av.number of fruit picked/wk	Average yield (kg)/week	Weight in kg/fruit	Diameter in mm/fruit
IL27	2	60-100	13.71 a	1.887 bc	0.1416 e	87.08 c
IL3	2	0-100	12.98 a	2.518 a	0.1929 cd	98.12 b
IL18	2.3	0-50/0-67	11.31 a	1.863 bc	0.1673 de	69.79 d
F1 with IL3	2	0-100	10.55 a	2.238 ab	0.2111 c	96.04 b
Variety control	_	_	7.91 b	2.195 ab	0.2736a	102.35 ab
IL10	7	0-25	7.69 b	1.911 bc	0.254ab	102.08 ab
IL13	4.9	75-100/75-100	7.6 b	1.878 bc	0.2504ab	99.37 b
F1 with IL 10	7	0-25	7.04 b	1.864 bc	0.2473ab	99.07b
IL 5	4	75-100	6.81 b	1.608 c	0.2282bc	98.54 b
Parental control	_	_	6.77 b	2.039 bc	0.2798a	108.75 a

Note: Chr. # and chr.part chromosome number and cM. - centiMorgan. Means separation within columns at genstat test of 0.05 levels. Lines with means difference not followed by the same letter are significantly different at p≤ 0.05.

Table 2. ILs chromosomes and parts and mean values for fruit development per line in days.

Genotype	Chromosome	Centimorgan	Fruit development rate/day
IL14	7,10	35 - 40,0 - 100	73.29 a
IL11	8	0 - 100	69.99 a
F1 with IL6	4	0 - 75	61.89 b
IL17	1b	0 - 100	61.68 b
IL5	4	75 - 100	61.48 bc
IL63	7	35 - 36	61.09 bc
IL19	6	20 - 100	60.9 bc
F1with IL16	12	0 - 67	60.47 bc
IL38	9	0 - 50	60.11 bc
IL6	4	0 - 75	60.11 bc
IL27	2	60 - 100	59.97 bc
IL45	11	75 - 100	59.71 bc
IL46	12	0 - 75	59.38 bc
IL15	11	60 - 100	58.84 bcd
IL20	7	40 - 100	58.53 bcd
IL22	12	95 - 100	58.41 cd
IL21	11	30 - 100	58.41 cd
IL51	10	0 - 100	58.41 cd
Variety control	_	_	58.27 cd
F1 with IL14	7,10	35 - 40,0 - 100	58.14 cd
IL18	2,3	0 - 50/0 - 65	58.03 cd
F1 with IL3	2	0 - 100	56.56 cd
F1 with IL12	9	0 - 67	56.17 cd
Parental control	_	_	55.78 de
IL3	2	0 - 100	54.95 def
IL16	12	0 - 90	52.95 defg
F1 with IL15	11	60 - 100	52.94 efg
F1with IL5	4	75 - 100	52.63 fg
F1 with IL11	8	0 - 100	52.34 fg
IL12	9	0 - 67	51.35 g
IL47	12	0 - 85	47.91 h

Mean separation within columns at genstat test of 0.001 level.
Lines with means difference not followed by the same letter are significantly different at p< 0.001.-Means no chromosome and part identified.

the fruit takes to grow into full size from fertilization through fruit setting to maturation. The flowering stage of all the lines was monitored. Flowers were labeled before they opened. Only flowers with very bright petals formed at the main shoot or from a branch

Table 3. ILs chromosomes and parts and mean values for fruit ripening per line in days.

Genotype	Chromosome	cM	Ripening in days/line
IL20	7	40-100	12.2 a
IL46	12	0-75	11.819 a
IL17	1b	0-100	11.633 ab
IL45	11	75-100	11.522 abc
IL18	2.3	0-50/0-65	11.5 abc
F1 with IL16	12	0-67	11.262 abcd
F1 with IL15	11	60-100	10.687 bcde
IL38	9	0-50	10.579 cdef
IL47	12	0-85	10.539 cdef
IL51	10	0-100	10.53 cdef
parental control	_	_	10.438 defg
IL22	12	95-100	10.2 efghi
IL12	9	0-67	10.12 efghi
IL3	2	0-100	10.071 efghi
variety control	_	_	10.07 efghi
IL16	12	0-67	9.923 efghij
F1 with IL12	9	0-67	9.913 efghij
F1 with IL5	4	75 - 100	9.847 efghij
F1 with IL14	7,10	35 - 40,0 - 100	9.818 efghij
IL63	7	35 - 36	9.8 efghij
F1 with IL3	2	0 - 100	9.761 efghij
IL19	6	20 - 100	9.725 efghijk
IL27	2	60 - 100	9.725 efghijk
IL15	11	60 - 100	9.583 fghijk
F1 with IL11	8	0 - 100	9.475 ghijk
IL21	11	30 - 100	9.4 hijk
IL14	7,10	35 - 40,0 - 100	9.361 ijk
F1 with IL6	4	0 - 75	9.212 ijk
IL5	4	75 - 100	8.969 jkl
IL11	8	0 - 100	8.717 kl
IL6	4	0 - 75	8.098 l

Mean separation within columns at Genstat test of 0. 001 level. cM means centiMorgan Lines with means difference not followed by the same letter are significantly different at P< 0.001. _Means no chromosome and part identified.

attached at the main shoot were labeled daily except on Fridays and through Sundays .The reason for labeling in that period was to be sure of the age of the fruit.

Fruit ripening

The same lines for fruit development were evaluated for fruit ripening under the same growing conditions. The lines are shown in the result (Table 3). Fruit ripening speed means the number of days it takes for the fruit green skin to change to red color. The fruits for all genotypes were evaluated for ripening speed. Fruits starting to ripe were tagged. Appearance of dark or bright green color on the fruit was the criterion sign used to determine the beginning of fruit ripening. Tagging of ripening fruit was done in the morning and checked the next early morning.

RESULTS

Fruit production

Production generally is difficult to determine because it is influenced by both genetic and environmental factors. The number of fruits collected per genotype weekly was recorded and analyzed statistically as indicated in (Table 1). Ripe fruits were collected for a period of 13 weeks. The highest production was in week 23 and the least was in week 16. The results showed a slight significant

difference ($p<0.001$) in fruit production among the lines. IL27 registered the highest mean production number of fruits. Conversely the parental control recorded the least production and differed significantly from IL3, IL18 and IL27 production rates. In heterozygous (different alleles control a particular trait) condition FI with IL3 was significantly different from the variety control production.

The total and unit yield in kg of the fruits of each genotype was taken immediately after harvesting. A slight significant difference exists in total yield among the genotypes. The IL3 is the only homozygous line (line in which the alleles controlling the trait like yield are identical) that differs significantly from the parental control. When the unit fruit weight was statistically analyzed per genotype, the results established that the weight of a variety control fruit was 0, 0625 kg heavier than the weight of the fruit collected from line FI with IL3. The weight of a parental control fruit was 0, 1382, 0, 1125, 0, 0869 kg heavier than a fruit collected from IL27, IL18 and IL3 respectively and weight difference was significant. The diameter of the biggest fruit per harvest for each line was measured in mm and the mean calculated after 13 weeks (Table 1). It is indicated that the biggest fruits were produced by the variety and parental control plants. Mean diameters of fruits from the parental plants were significantly different from IL13, IL5, IL3, IL18 and IL27. The size of a parent fruit was almost 1.5 times the size of a fruit produced by an IL18 plant.

Fruit development

Fruits investigated for development speed were collected from five plants per genotype and the data tested (Table 2). The genotypes had two year culture. The ILs was evaluated for both development and ripening. The ILs high - lighted in red had been tested before and included in this trial for fine mapping and confirmation and the 6 last ones shown in purple were new and had not been tested earlier. The rest had been investigated in previous trials.

The results in Table 2 showed evidence of significant differences in the development speed among the lines ($P<.001$). Significant difference was found between the parental control and 13 genotypes. Fruits from the parental control were 8 and 4 days slower in developing than fruits collected from IL47 and IL12 but faster than the remaining 11 lines. No significant difference was observed between the variety control and the lines (F1 with IL5, F1 with IL15, F1 with IL12, F1 with IL3, F1 with IL14, F1 with IL 16 except in F1 with IL6 and F1 with IL11). IL 47 and IL12 showed significant different effects while IL16 and IL3 had intermediate effects compared to the parent control fruits. Lines IL18, IL51, IL21, IL22 and IL20 were slightly slower in speed but not significantly different from the parent line. Fruit picked from the remaining twelve lines were slower compared to the fruits of the parent line in development. Only fruits from F1 with IL11 and F1 with IL6 differed significantly from the variety control fruits in development rate

Fruit ripening

The fruits collected from P50 were analyzed for ripening speeds. The variety control significantly differed from the F1 with IL16,IL5,IL6 and IL11 The lines IL6, IL11, IL5, IL14,, IL21 had a faster ripening speed and conversely IL18, IL45, IL17, IL46 and IL20 were slower in coloring speed than the fruits collected from the parental control plants in Table 3. In P49, significant differences in coloring speed were found among the lines (F-prob<0.001). IL 27 was faster (2 days ahead) line other compared to the parental control line. Fruits from the genotypes had intermediate ripening rate. All the heterozygous lines(lines in which the alleles that control the traits are different) had recessive effect except F1 with IL10, which registered a dominant effect in relation to the speed the variety fruits took to complete coloring.

DISCUSSION

Fruit production

The results are compared in the discussion to our preliminary trial results (some) we obtained for the precise mapping of the traits on the 12 chromosomes of the pepper lines. Genetic analysis of yield in interspecific crosses is said to be affected by some overshadowing QTL associated with partial sterility (Eshed and Zamir, 1995). In this trial no sign of plant with sterility was observed. However, a variation in the number of fruit production was apparent. IL27, IL3, IL18 and F1 with IL3 (Table 1) produced the highest number of fruits compared to their control lines.

As expected IL3 is among the lines with the highest production. On the contrary IL13 had relatively lower fruit number though its total yield was not significantly different from the parental control. IL18, IL3 and IL27 differed completely from the parental control with respect to the number of fruits produced. The variety control was slightly higher than F1 with IL3 in terms of the number of fruits produced and the difference was significant. This suggests the F1 with IL3 had an incomplete dominant effect. Similar research indicated that the QTLs that influence fruit number, fruit weight (size) and yield are in chromosomes 2, 3, 4, 8, 10 and 11, Ben-Chaim et al. (2000), Rao et al. (2003). Anne Frary et al. (2000) and Zygier et al. (2005) detected in the genomic region Fw 2.1 and Fw 2.2 of chromosome 2 to be the locus that affects fruit size in pepper and tomato. Our earlier results obtained in 2003 found that chromosome 2 (0-100 cM) in IL3 and chromosome 4 or 10 (75 -100 cM) in IL13 had the highest production in the number of fruits. In this research 2006, the highest production of fruits in

numbers occurred in ILs 27 and 3 in chromosome 2(60 - 100 cM) and in IL18 in chromosome (0 - 50 cM). The 2006 results do agree that QTLs for higher of fruits produced are in IL3 in chromosome 2 in the part 0 -100 cM.

Fruit development

Fruits of F1 with IL11 and F1 with IL6 were almost 6 days ahead and 4 days behind the control fruits in development rate respectively. This strongly indicates that the effects of these introgression segments (IL11 and IL6) in these heterozygous lines are dominant. All other heterozygous lines had recessive effect when compared to the variety control line. In contrast, IL18 and IL5 found to have fruits with slower fruit development in P50 were among the fastest lines with fruits that developed so fast in P49 in relation to the parent control line. The reason for this discrepancy may be that the fruits were at different developmental stages. The behavior of IL27 in both glasshouses was similar. The fruits from IL13 and IL18 differed significantly in development rate (F-prob<0.001) from IL3, IL27 and the parent control line. The developmental speed effects on fruits of these two lines were significantly fast. The two heterozygous lines (F1 with IL10 and F1 with IL3) were quite slower and had dominant effect than the variety control and differed significantly in fruit development. These results confirmed lines IL3 and IL16 previously identified as lines with traits for faster fruit development. However, effects contrary to expectations based on earlier results were seen for lines IL14, IL6 and IL15 which had slower fruit development. Initially lines in P49 were only meant to be tested for production, perhaps that was the reason, IL14, IL6 and IL15 were excluded from the trial in that glasshouse. According to the analysis IL47, IL12, IL13 and IL18 are newly discovered genotypes for faster fruit development.

The QTLs that enhance fruit development were identified and mapped on chromosomes 2, 12, 7or10, 4, 11 (0 -100, 0 - 90, 35 - 40, 0 -75 60 -100) cM respectively in 2003. In 2006 (Table 2) the QTLs for fruit development were found in the parts between 0 -50 cM in chromosome 2, 0 - 85 cM in chromosome 12, 0 - 67 cM in chromosome 9, 0 -100 cM in chromosome 8 and in 75 - 100 cM chromosome 4 .Thus the hypothesis that the traits for fruit development may be in chromosomes 2, 12, 7or 10 and 11 on the (0 -100, 0 - 90, 35 - 40 and 60 -100) cM parts is rejected.

Fruit ripening

Fruit ripening is signaled by the disappearance of the green color on the flesh of the fruit. It is completed when 95 - 100% of the fruit is red. The period from seed germination to the beginning of fruit ripening can range from 120-128 days. Several factors such as enzymes (Jimenez et al., 2002), delayed breakdown of chroloplast structure (Hornero-Mendez and Minguez-Mosquera, 2002), ethephon application (Armitage,1989; Kahn,1992; Cooksey et al., 1994), Y+ dominant allele genes (Lefebvre et al., 1998), ethylene production (Tadesse et al., 2002; Pretel et al., 1995), capsanthin and zeaxanthin substances (Abellan - Palazon et al., 2001) and vitamin C (Pillai and Abraham, 1996) have been implicated in influencing fruit ripening. In this project, it was observed in both glasshouses that fruits formed at the upper part of the mother plants were faster in coloring than those at the bottom between periods. Probably the fruits at the top received more radiation as the day length and the average greenhouse temperature increase from spring to summer but this cannot be verified as there was no radiation measurement taken. The lines IL27, F1 with IL3, IL11, IL14, IL6 and F1 with IL6 with slow fruit development had faster ripening speed. It is very likely that the size of the fruits plays effective role in the ripening process. As expected IL11 and IL5 had similar ripening speed as reported in the previous research we conducted. However these results found that IL6 and IL27 produced fruits with the fastest coloring speed In P50. But the results of 2003 suggested that IL6 was faster in fruit development rather than coloring speed. No tangible explanation was found for this difference though 2003 trial was conducted in the same season and under similar circumstances but there is an indication that the speed inheritance for development is recessive.

The QTLs that induce fruit ripening are reported to be located in chromosomes 2, 3, 7 and 8 (Ben-Chaim et al., 2000). In 2003 mapping, our research team mapped the QTLs in chromosomes 4 (75-100 cM), 8 (0-100 cM) and 9 (0 - 65 cM). This 2006 results showed that the QTL for quick fruit coloring are located in chromosomes 4 (75 - 100 cM) in IL5, chromosome 8 (0-100 cM) in IL1, chromosome 2 (60 -100 cM) in IL3. The results are partly in agreement with Ben-Chaim's and our 2003 results in relation to the presence of the QTLs for ripening trait in chromosomes 8 and 4. Fruits harvested from lines IL6, IL11, IL5 IL14 and IL21 in P50 are faster in coloring speed than the parental control fruits. The difference in speed between them was significant (F – prob < 0.001). Five of the homozygous lines were slower in ripening than the control. The remaining 10 lines demonstrated equal and intermediate ripening speed effect in comparison to the control. F1 with IL16 was the only line significantly different from the fruits collected from the variety control line. Its fruits took 2 days more to finish coloration than the variety control fruits. All other tested heterozygous lines showed equal ripening speed effect on the fruits. From the above discussion, there are clear evidences to suggest that the introgression line segment did not have dominant effect on the ripening speed, resulting in an effect in the F1 IL fruits. But there are 10 cases where, the homozygous lines produced fruits that

had significant differences in ripening speed from the original parental line.

We conclude that IL3 chromosome segment (0 -100 cM) contains the trait for higher production and total fruit weight. The trait was also fine mapped at chromosome 2 at the first (0 - 50 cM) and the last segments 60 -100 cM for IL18 and IL 27 respectively. We are unable to confirm that the QTLs for fruit development are in chromosomes 2, 4, 9, 8 and 12 as our present findings differ with 2003 results. However we confirmed and fine mapped the fruit development trait on the chromosome 2 part 0 - 50 cM and chromosome 3 part 0 - 65 cM. The pattern in development of fruits in IL16 is in total disagreement with fruits in IL47 even though both lines have the same chromosome and contain almost the same segment size. The cause of this discrepancy requires further investigation. The ILs 5 and 11 results confirmed earlier conclusions that the fruit ripening trait is at chromosome 4 and 8 at 75 -100 cM and 0 -100 cM, respectively. The genotype IL14 had a combination of a small and large segment from chromosomes 7 and 10. The locus for the ripening trait may be in either of the two segments. In order to fine map the trait, it will be necessary to do backcrossing of the line, select with markers and do selfing so as to separate the combination.

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