דוח לתוכנית מחקר מספר 261-0726-10

שילוב גנטיקה וגנומיקה ללימוד QTL בעגבניה

Integrating genetics and high throughput genomics to identify genes underlying tomato QTL for metabolites that influence fruit quality

מוגש לקרן המדען הראשי במשרד החקלאות

ע"י

ארי שפר, אילן לוין, ילנה יסלסון, מרינה פטריקוב, שמואל שן, סעדיה נהון, לאה חן, חביבה שלמה

המחלקה לחקר ירקות, מינהל המחקר החקלאי

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הממצאים בדו"ח זה הינם תוצאות ניסויים. הניסויים לא מהווים המלצות לחקלאים

חתימת החוקר _____

תקציר :

הצלחנו בשנה השלישית להשיג את מטרות התוכנית. ביצענו אנליזה טרנסקרופטומית על פירות של קוים הקובע היחס בין פרוקטוז לגלוקוז בפרי והגדרנו את גבולות המחדר. צמחים FGRכמעט אקזוגניים לגן טרנסגניים לאחד המועמדים הראו שהגן הקובע את התכונה הוא נמצא על ידו וצמחים טרנסגניים למועמד FGRהשני נמצאים בשלבי הכנה. גם פיתחנו אוכלוסיות וסמנים למיפוי העדין של גם מודיפיאר ל ואוכלוסיית המיפוי נמצאים עכשיו בבדיקה. מיפינו כ50 גנים של מטבוליזם של חומצות אורגניות . גם והפירות הועברו לשותפים באנגליה PIMPINELLIFOLIUM המבוסס על מין הבר RILגידלנו אוכלוסיית לבדיקת ההרכב המטבולי של האוכלוסייה.

Summary final report by the coordinator, Prof. Graham Seymour, University of Nottingham

Fruits are an important part of the human diet. Low fruit and vegetable intake is recognised as a major factor for increased risk of heart disease and certain cancers. Quality and health attributes are linked to molecular and biochemical changes in fruits during development and ripening. Some of the most important changes in fruit quality involve the accumulation of primary and secondary such as sugars and acids which influence taste and carotenoids and phenylpropanoids that impact nutritional quality. Tomato is the most widely consumed fruit, with a global value in excess of $30 bn. It has become the model for studying the development and ripening of fleshy fruits and a wealth of genetic and genomic resources are available. The aim of the project was to understand the molecular mechanisms controlling the formation of primary and secondary metabolites responsible for tomato fruit quality traits and harness natural variation for the benefit of the European consumer. The multinational consortium which included a global biotechnology company targeted quantitative trait loci (QTL) involved in primary and secondary metabolite accumulation using new micro introgression lines from wild tomato species genetic resources and a range of tomato mutants and transgenic approaches.

Genes controlling sugar metabolism and especially the relationship between fructose and glucose levels in tomato were major targets with the aim being to enhance sweetness by elevating fructose or sucrose levels. A candidate sugar transporter gene underlying an *S. pennellii* QTL controlling fruit fructose / glucose ratio on Chr 4 was identified. Also another fructose / glucose ratio QTL on Chr 6 and a sucrose accumulator on Chr 12 were fine mapped. Malate was shown to play a crucial role in tomato starch metabolism and be important in postharvest properties of fruits. A tomato malate QTL on Chr 3 was resolved and metabolite analysis continues for candidate gene nomination. Another focus was the accumulation of branched chain amino acids (BCAA) in tomato that are essential components of the human diet and involved in volatile flavour generation. This involved characterisation of the BCAA transferase gene family and identification and functional testing of an isopropylmalate dehydrogenase underlying a BCAA QTL.

Tomato fruit contain a wealth of secondary metabolites that are important in a healthy diet and protect against cardiovascular disease and certain cancers. A novel lycopene QTL on Chr 2 was resolved and candidate genes identified by expression analysis. Additional candidate genes under new QTLs for beta-carotene and alpha-tocopherol have also been identified. The

project allowed preliminary investigation of substantially enhanced pigment composition of transgenic fruits over expressing regulatory gene linked to *S.pennellii* QTL on Chr 8. A pepper ortholog was identified by the industrial partner. Furthermore comprehensive metabolite database for the *S.pennellii* ILs was generated. The project provided insights into the links between metabolite changes and the higher regulatory network controlling ripening in fruits. The role of the AP2 transcription factor in ripening and its effect on metabolism was established and gene regulatory networks constructed by the partners are now being used to link AP2 to other major ripening transcription factors.

The validation of candidate genes underlying QTL which were identified has begun and the project facilitated development of a Micro-Tom mutant collection and TILLING platform. Candidate gene validation will now continue in a follow-on BBSRC LINK project. Outputs from the work will be of direct benefit to the consumer because several of the metabolite QTL regions are being introgressed into commercial lines by the industrial partner and translation to other fleshy fruit has identified alleles in pepper (sweet and hot) for colour intensity and retention.

BBSRC Final Report Questionnaire reorganise as / summary

1. What were the most significant achievements from this grant?
   (up to three, no more than 150 words in total).

Achievements for UoN led tasks within context of larger consortium

1. 113 Chr 2 and 41 Chr 3 marker defined QTL-NILs generated and fruits analysed for metabolomics by other partners. These resources facilitated UoN / Syngenta fruit quality patent (WO2011/051120 AI) and resolution of an important quality QTL Chapman *et al*, (2012).

2. With RHUL, novel lycopene QTL on Chr 2 resolved to 111 kb and candidate gene identified by expression analysis. With Max Planck malate QTL resolved on Chr 3 and metabolite analysis being completed for candidate gene nomination.

3. Substantially enhanced pigment composition of transgenic fruits over expressing regulatory gene from UoN validated by RHUL and linked to an *S.pennellii* QTL on Chr 8. A pepper ortholog was also identified with Syngenta. Joint outputs with RHUL partner were an international patent application WO2012041856 and building on ERANET resources a follow on BBSRC / Syngenta LINK project total value £1.2 M. MS submitted to *Plant Physiology*.

Additional major achievements of consortium: (300 words)

1. A sugar transporter gene underlying a fructose / glucose ratio QTL on Chr 4 was identified by

the Volcani partner. They also fine mapped further fructose glucose ratio QTL on Chr 6 and a sucrose accumulator on Chr 12 using alleles from various tomato wild species (Publications in preparation).

2. The tomato branched-chain amino acid (BCAA) transferase gene family was characterised by the Max Planck partner. They identified and functionally validated an isopropylmalate dehydrogenase underlying a BCAA QTL (Kochevenko and Fernie, 2011; *J. Exp. Bot*; Kochevenko, et al, 2012. *J. Plant Phys*; Kochevenko, et al, 2012; *Mol Plant*).

3. Identification of candidate genes under QTLs for tocopherol and β-carotene and generation of a comprehensive metabolite database for the *S.pennellii* ILs including construction of correlation network across metabolism and examples focussing on health related isoprenoids formed in tomato fruit by RHUL and UoN partners (Wells et al., 2012. PNAS Submitted for publication).

4. The role of the AP2 transcription factor and its effect on metabolism has been established by the Wageningen partner working also with RHUL and Max Planck (Karlova et al, 2011. *Plant Cell*). UoN, RHUL, Syngenta GRN now being used by UoN and Wageningen to link AP2 to other major ripening Tfs (In preparation).

5. Project has facilitated development of Micro-Tom mutant collection and TILLING platform belonging to INRA partner. TILLING identified mutant lines in 1-hydroxy-2-methyl-2-(E)-butenyl 4-phosphate synthase, geranylgeranyl pyrophosphate synthase 1 and 2, Lin5, mitochondrial malate dehydrogenase, AP2, FUL1, FUL2 and HFP.

6. Syngenta are taking several of the metabolite QTL regions through their gene factory to introgress wild species alleles into commercial lines, e.g beta carotene and flavonoids, with the objective of introducing these traits into commercial practice. Translation to other fleshy fruit has identified alleles in pepper (sweet and hot) for colour intensity and retention.

2. Indicate whether or not the main objectives of this grant were met and, for any which were not met, provide a brief explanation (no more than an average of 50 words for each, no more than 300 words in total).

Main objective was to identify candidate genes under a range of metabolite QTL. This has been achieved. Several candidate genes need to be validated in transgenic plants. Due to the success of TomQML, RHUL and UoN have a follow on LINK grant from Syngenta that will allow selected experiments to be completed.

There are still a number of additional publications and patents to be delivered from this project and these are in preparation. It is important to note, that working with crops, e.g. tomato, which often have a relatively long life cycle in comparison to model systems, almost always means that within the time frame of a standard three years project experimental work can be completed, but then a further 12 months is often required to finish IP capture and subsequent manuscript submission.

The project added to the metabolite profiling expertise in tomato and will substantially extend existing databases at SGN (http://solgenomics.net/), the international repository for Solanaceae-related data.

סכום

מטרות המחקר לתקופת הדו"ח תוך התייחסות לתוכנית העבודה.
לשבט את הגן FGR, ולהכין דוגמאות של פרי עגבנייה מאוכלוסיית RIL, למפות הגנים של מטבוליזם של חומצות אורגניות ולבצע מיפוי עדין של הגן MO-FGR
עיקרי הניסויים והתוצאות שהושגו בתקופה אליה מתייחס הדו"ח.
הצלחנו לשבט את FGR וגם להכין צמחים טרנסגניים המבטאים בעודף את הגן, לבצע את ניסוי ודיגום של אוכלוסיית RIL המבוסס על המין PIMPINELLIFOLIUM.
המסקנות המדעיות וההשלכות לגבי יישום המחקר והמשכו. האם הושגו מטרות המחקר בתקופת הדו"ח.
כמעט כל המטרות הושגו. עדיין מחכים לתוצאות של צמחים טרנסגניים ולתוצאות מטבולומיות מהשותפים האירופאים.
הבעיות שנותרו לפתרון ו/או השינויים שחלו במהלך העבודה (טכנולוגיים, שיווקיים ואחרים); התייחסות המשך המחקר לגביהן, האם יושגו מטרות המחקר בתקופה שנותרה לביצוע תוכנית המחקר. מכיוון שהשותפים באירופה התחילו את הפרוייקט באיחור חלק מהתוצאות (המטבולומיקה) עדיין לא מוכנות.
. האם הוחל כבר בהפצת הידע שנוצר בתקופת הדו"ח - <u>יש לפרט</u>: פרסומים – כמקובל בביבליוגרפיה, **פטנטים - יש לציין מס' פטנט, הרצאות וימי עיון - יש לפרט מקום ותאריך.**
היתה הרצאת התקדמות בפגישה החצי שנתי של השותפים, בישראל במאי 2011 וגם באנגליה במאי 2012. המאמר בנושא FGR יוגש לפרסום מיד עם בדיקת הצמחים הטרנזגניים.
פרסום הדו"ח: אני ממליץ לפרסם את הדו"ח: (סמן אחת מהאופציות)
➢ לא לפרסום מכיוון שחלק מהשותפים לתוכנית הם חוקרים באוניברסיטאות באירופה ויש מעורבות גם של חברה עסקית (SYNGENTA) ואין רשות מהם לפרסם התוצאות.