Project acronym: PROTEIN2FOOD

Project No.: 635727

H2020-SFS-2014-2015/H2020-SFS-2014-2

Start date of project: March 2015

Duration: 5 years

Deliverable reference number and title

D1.9 Transcriptomes for developing SNP markers for selected adaptability traits

Date: 28.02.2019

Organisation name of lead for this deliverable:

Swedish University of Agricultural Sciences (SLU)

	Project co-funded by the European Commission within the Horizon 2020 Programme	
	Dissemination Level	
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

0. Summary

For the future plant breeding of faba bean to become more efficient, the development of molecular breeding tools is needed. Faba bean can already today grow in diverse climatic environments around the world, but one challenge this crop faces in the Northern European environments is the long growing season of the crop compared to several other species. This is a factor today limiting the expansion of faba bean acreages further north. The aim of this study was to characterise and identify candidate genotypes for transcriptome analyses for the development of SNP markers. The aim is to then use these markers in breeding programs to develop new early faba bean varieties that are better adapted to Northern European environments. For this purpose, we investigated earliness traits in a set of genotypes under greenhouse conditions, including both older breeding material from North Europe available from NordGen (Alnarp, Sweden) as well as modern cultivars. Relatively large genotype differences were found, and candidate genotypes suitable for transcriptome analyses are suggested in the deliverable.

1. Introduction and objectives

One of the main objectives of Protein2Food is to increase both the growth and use of protein crops in Europe. Work Package 1 (WP1), 'Crop Production', is aiming to improve the knowledge on cultivar improvement and crop management for protein crops in European environments. To be able to improve the target crops we need to identify trait variation in this crop, which can be combined through plant breeding in aim to yield new improved cultivars. Transcriptomes (i.e. sequences of all genes used in a plant tissue at a certain time point) of genotypes that show variation in a specific trait can be used to understand the genetic basis for this trait. This information can be further used to develop genetic markers such as single nucleotide polymorphism SNPs (Filali et al., 2016; Kumar et al., 2012) that can facilitate future breeding programs.

Faba bean (*Vicia faba*), can be regarded an orphan crop with regards to the absence of modern breeding tools, such as genetic molecular markers, even though the situation is starting to change (Maalouf et al., 2017; O'Sullivan and Angra, 2016). Increasing production area and use of this crop in both food and feed in Europe, provides a high potential for faba bean contributing to the increasing self-sufficiency of plant based protein production in Europe. This, particularly due to the high yield potential and high protein content of faba bean, compared to other legume crops. However, the present faba bean cultivars often suffer from yield instability. This is thought to partly be explained by its flowering and growth habits, i.e. profligate flowering and indeterminate growth. These provide many flowers over many nodes, thus resulting in a long flowering and pod set periods, which can have a negative impact on yield (O'Sullivan and Angra, 2016).

The objective in this deliverable was to identify genotype differences in earliness traits in faba bean, which could be of interest for transcriptome studies and contribute to the development of breeding tools for future breeding programs. Faba bean is a crop that is rather compatible with North European environments. However, the crop has a long growing season and therefore earliness traits are of high importance. 'Earliness' in faba bean can be seen as an integration of several different traits, such as early onset of flowering, a determinate flowering and pod set period, and early maturation of pods. The timing of flowering is part of the phenological development of a plant in which the energy allocation between vegetative and generative growth needs to be balanced. The breeding of 'topless' or determinate faba bean varieties over the last few decades have resulted in a decreased height (with flowering ending at the 3rd of 5th node), increased lodging resistance and earliness of the crop, which facilitated crop management and harvest (Stelling, 1997).

Available knowledge on genes determining growth habits in other plant species, has allowed the development of molecular markers that determine growth in faba bean, however these have showed some predicted inconsistencies in different germplasm populations (Torres et al., 2010). Moreover, the determinant varieties often suffer from lower yield potential compared to indeterminate varieties. Therefore, more knowledge on the genetic regulation between vegetative and generative stages in faba bean is needed. This includes knowledge on the onset of flowering to identify candidate genes and markers to be included in future breeding programs on faba bean (Patrick and Stoddard, 2010). In the long term, this knowledge is wished to contribute in expanding the production acreages of faba bean into Northern European environments.

2. Activities for solving the task(s)

Eleven different faba bean genotypes were grown and screened for flowering (onset of flowering in time after sowing, and following which node numbers were flowering during plant development) and growth behavior (height of plants and branching frequency during plant development) under greenhouse conditions during February-June 2018 (at SLU in Alnarp, Sweden). The genotypes included modern cultivars used in Europe today (Fanfare and Taifun) as well as older cultivars and breeding material that were accessed through the gene bank NordGen (Alnarp Sweden). Among these genotypes, Pihronen was previously used in faba bean breeding programs as a donor of 'early traits'- even though this genotype has other non-desired traits from an agronomic perspective, such as low yield (personal communication, Jan Sjödin, previously breeder of faba bean at Svalöf Weibull, Sweden). Mikko and Kontu are two cultivars from a breeding program from Finland (released 1982 and 1990, respectively), aiming at combining earliness with acceptable yield and N fixation levels (Stoddard et al., 2009). Other varieties applied are from Swedish breeding programs during 1930-1990, many which focused mainly on seed size (Primus, Sving, Vega, Aurora) and to some extent earliness (Arla).

The temperature set points for warming and venting in the greenhouse were 18°C and 21°C, respectively, and the natural light was supplemented with artificial light when below 200W, with a day/night regime of 16h/8h. It should be noted that the temperature were often higher than 21°C during warm spring/summer days when the venting system was not enough to keep the temperatures low. To minimize the impact of differences in light conditions within the greenhouse chamber, the plants position in the chamber were rotated three times a week. No fertilization was applied (legume crops fix nitrogen through Rhizobium bacteria symbiosis; all plants developed nodules in their root system which was checked after the screening), and the micronutrients available in the potting soil used were estimated to be enough for the whole growth period.

Because faba bean is a species with relatively high outcrossing frequency (i.e. needs pollination to develop seeds), plants were manually pollinated using a brush three times a week. Manual pollination, however, is not as efficient as natural pollination done by insects. Therefore, no reliable meaningful seed yield data could be achieved from our study, but instead the focus was on characterizing the flowering and growth behavior during plant development. It could be noted that in the field conditions, the days from sowing to onset of flowering in field would be much higher (due to a lower temperature), but the purpose from our greenhouse trial was to reveal any large genotype differences.

3. Results

Relatively large genotype differences could be observed in flowering behavior amongst the screened genotypes (Figure 1). The genotypes with the earliest onset of flowering were Pihronen, Kontu, Arla and Mikko, which started to flower 36 days after sowing, while the latest flowering genotype, Aurora, started to flower 51 days after sowing. The late onset of flowering of Aurora could possibly be a result from a slow establishment (growth) if looking at height of plants (see below). However, the targeted breeding goal for this variety was seed size (and not earliness), It is, therefore, possible that Aurora might be a true late flowering variety. The two modern cultivars included in the study, Taifun and Fanfare, started to flower at 42 and 48 days after sowing, respectively. The early onset of flowering of Kontu, Arla and Mikko was in agreement with the described breeding goals during development of these varieties, as well as Pihronen described to be a 'donor' of earliness genes.

The time span of the flowering period was different between genotypes, for example Arla had a very short flowering span and with several other genotypes the flowering period was never ending within the screening time under these greenhouse conditions. The amount of flowering nodes on one plant at the same time also differed between genotypes, with Arla having a spatially more condensed flowering with only four nodes as a maximum number, while others had six.
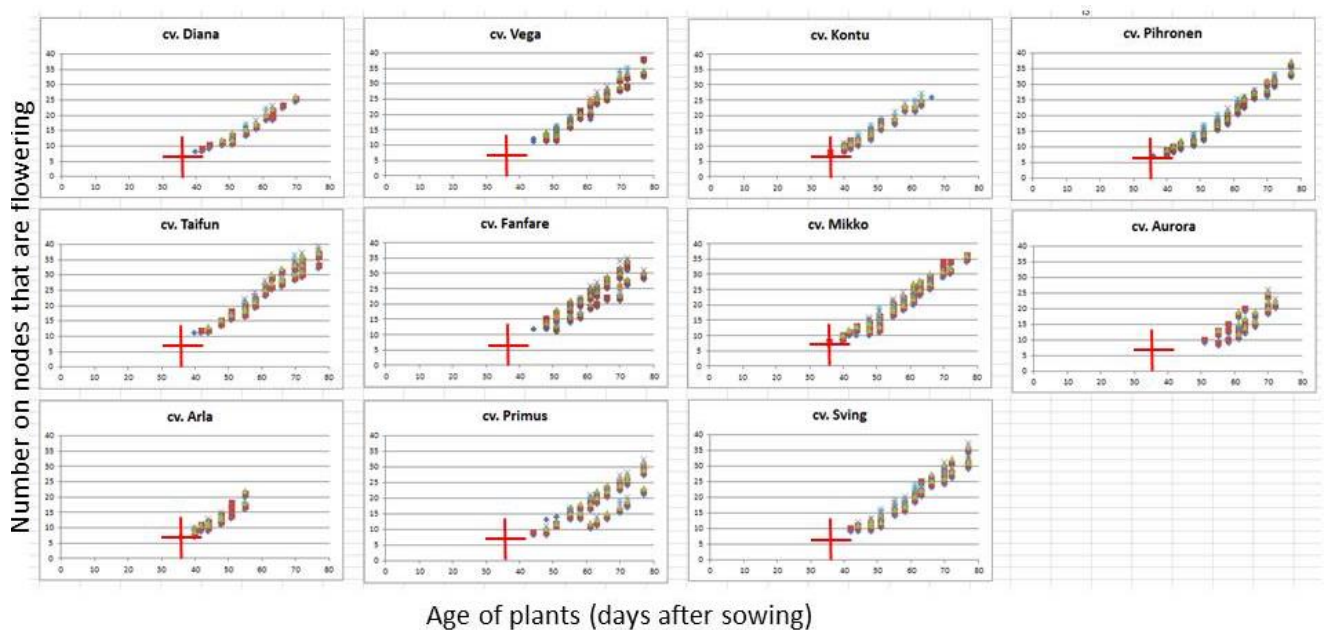
Figure 1. Flowering behavior of different faba bean genotypes. *Data given are observations from four biological replicates (four individual plants) grown under greenhouse conditions (SLU, Alnarp, Sweden). Each node that was flowering on each plant was noted and plotted against the age of the plant. The red cross marks the intersection of the first time point of flowering (36 days after sowing) and the lowest number on first flowering node (no 7) found in the set of genotypes screened, to allow easier comparison between the genotypes.*

Height of plants and branching were measured to identify differences in vegetative growth behavior (Figure 2 and 3, respectively), which showed significant genotype differences. For example, Diana and Kontu indicated a growth behavior of determinate growth (i.e. stopped growing), whilst most other genotypes in fact never stopped growing under these greenhouse conditions (Figure 2). Due to this fact, the plants also had difficulties to enter into maturation of developing pods without having to cut off the top of plants. Therefore, no data on time from sowing to maturity was taken. Branching behavior was also different between the genotypes. Aurora for example had very few branches, whilst the genotypes Pihronen and Mikko had significantly more (Figure 3). The modern cultivars Taifun and Fanfare had a relatively low degree of branching. However, no obvious correlations between onset of flowering to height and branching of plants were observed in our study.
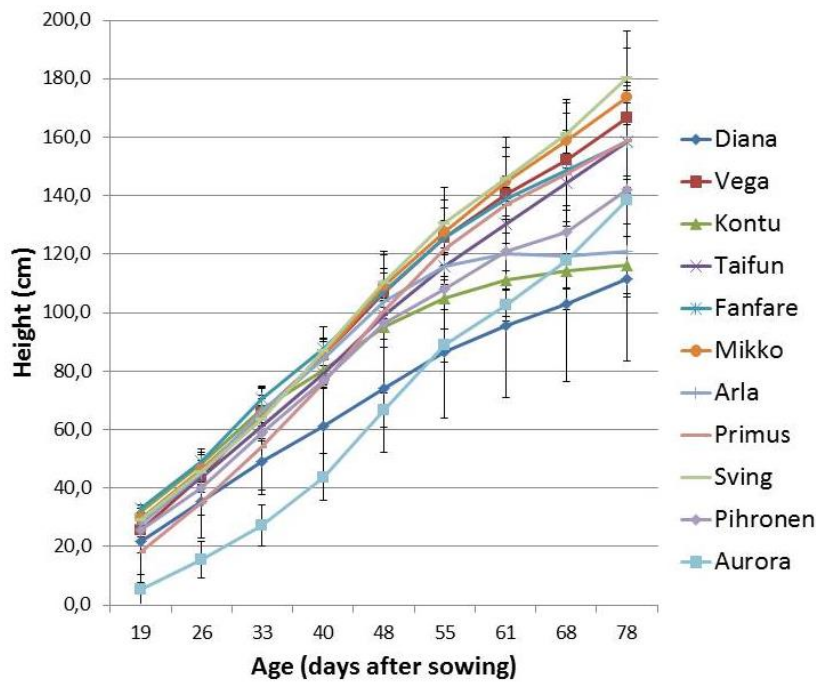
Figure 2. Height differences recorded for the faba bean genotypes. Data given are mean values from four biological replicates ±standard deviation.
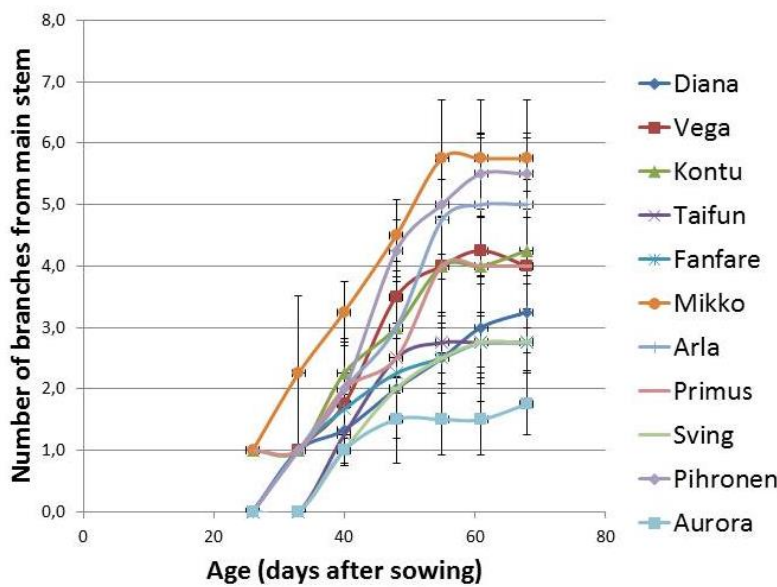

Figure 3. Branching differences recorded for the faba bean genotypes. Branching is given as number of branches >5cm appearing from main stem. Data given are mean values from four biological replicates ±standard deviation.

4. Conclusion and next steps

The aim of this study was to characterise and identify candidate genotypes for transcriptome analyses for the development of SNP markers targeting earliness traits in faba bean. These could be used to develop new early varieties that are better adapted to Northern European environments. For this purpose we investigated earliness traits and connected growth habits in a set of genotypes, including both older breeding material from North Europe and modern cultivars.

Genotype differences were identified in flowering and growth behavior in the faba bean genotypes was analysed, suggesting that this material can be of interest for in-depth transcriptome studies. The onset of flowering was earliest in Pihronen (previously used as a 'donor' for earliness genes in breeding programs) and in three more varieties of Kontu, Arla and Mikko (all from previous breeding programs in Finland). Among the investigated varieties Aurora (from previous Swedish breeding program) showed the latest onset of flowering, while Taifun and Fanfare (modern cultivars from German breeding programs) were in between. Candidates for transcriptome studies, in attempt to identify markers for early flowering, should include the most diverse varieties. Therefore, Pihronen (taking into account its early onset of flowering and its use previously as a 'donor' in breeding programs) and Aurora could be suitable varieties. However, before performing such transcriptome analyses, the genotype differences observed under greenhouse conditions should first be confirmed under field conditions.

5. Delays and difficulties

In WP1, quinoa and faba bean were selected as target crops for the detailed molecular studies as representatives of protein crops with high protein quality and quantity, respectively. Both these traits are of importance for the increased production and use of these crops for food purposes in Europe. Due to budget limitations for RNA-sequencing in WP1, RNA-sequencing (transcriptomes) of quinoa was prioritized due to the work done for D1.4 and D1.5. Therefore, this study (D1.9) was limited to identify candidate germ plasm of faba bean for adaptive traits.

One challenge with faba bean is that it is a partly cross-pollinating crop which needs pollination to develop seeds. Therefore, under greenhouse conditions manual pollination is often required to develop seeds (even though genotype differences occur). This is, however, not as efficient as natural pollination done by insects. This resulted in the fact that no reliable or meaningful seed yield data could be achieved from the performed study. Therefore, the study was focused on the flowering and growth behavior occurring during plant development and affecting the yield. On the other hand, under field conditions the naturally occurring pollination might cause genotype mixing, especially if small plots are used, which would make screening of many genotypes challenging.

6. Impact and outreach

In most parts of Europe, faba bean is widely used today for animal feed. However, increasing the availability of farmers for new improved crop varieties that can adopt in more diverse climate conditions and are known for its high quality usage in food, such as faba bean, could assist in reaching the full production and market potential of such crops in Europe (Comission, 2018). The domesticated gene pool of faba bean show a large variation in many different aspects (Duc et al., 2010), and the crop is already grown in parts of Northern Europe. However, new cultivars that flowers and reach maturity earlier would make it possible to expand the acreages of this crop in Northern Europe. More diversified agriculture in Europe, for example done by expanding acreages of legume crop production, would furthermore benefit from the nitrogen-fixing legume crops in the agricultural systems. This study on the characterisation and identification of genotypes that differ in flowering behavior can be of interest for further transcriptome studies in developing genetic markers to be used in future plant breeding programs of faba bean.

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