Project number: 5769 (B)
Funding source: RSF 07 501

Date: November, 2012
Project dates: Dec 2007 – Dec 2012

Physiological and genetic response of maize to low temperature conditions

Key external stakeholders:
Maize breeders, agronomists, plant science research community

Practical implications for stakeholders:
The outcome of this research informs agronomists and breeders on the impact of cold stress on the performance of maize varieties at early developmental stages.

- For both root and shoot tissues the effect of cold stress on the growth of a variety varies depending on the time of treatment. The differential responses of the varieties could not be explained by either kernel type or maturing time, however, the two varieties with the highest level of cold tolerance were very early maturing, flint types.
- From the 46K maize array, genes involved in chilling tolerance were identified. These could be targets for the improvement of cold tolerance of varieties.

Main results:
In the physiological experiments the genotypes presented a wide range of cold tolerance, which was dependant on the time point of the cold treatment.
The analysis of the microarray showed significant changes in gene expression between controlled conditions and cold treatment for the cold tolerant varieties studied, but not for the cold sensitive varieties. These differentially expressed transcripts could be followed up in marker assisted breeding programmes for the development of cold tolerant varieties.

Opportunity / Benefit:
Maize is a desirable crop for the production of high quality animal feed. Due to its origin in warmer climates the crop is not well adjusted to cooler temperate climates. Progress has been made to develop novel maize cultivars better adapted to temperate climates, especially in terms of tolerance to cold spring temperatures and chilling shocks. This project examined the genetic causes of cold tolerance/susceptibility of a range of maize cultivars. An improved understanding of the underlying causes of cold tolerance can help in the production of better adapted maize varieties.

Collaborating Institutions:
UCD Dublin

Contact Susanne Barth Email: susanne.barth@teagasc.ie.

Teagasc project team: Dr. Susanne Barth (PI)
Mauro di Fenza

External collaborators: Prof. Thomas F. Gallagher, UCD

1. Project background:

This project is part of a multidisciplinary project with the aim of providing a functional evaluation of the potential of maize (*Zea mays*) for use in low-input, sustainable production systems that are better suited to temperate conditions. But also to provide a basis for the further exploitation of maize as an alternative, more widely used fodder and bioenergy crop. A wide range of maize varieties and breeding lines were examined in laboratory-based experiments using comparative genomics, supported by physiological methods of assessing plant performance. A particular focus was placed on the impact of low soil temperatures during early seedling establishment. A detailed genetic study of the genes involved in cold stress tolerance in maize has been made available by a publicly accessible 46K maize array. The development of maize hybrids able to grow with high performance at low temperatures would result in improved establishment of the crop in cool climate regions like Ireland.

2. Questions addressed by the project:

- How large are varietal differences in the response to chilling shock in root and shoot tissues of maize?
- Can we identify the major genes underlying cold tolerance using a high density maize microarray?

3. The experimental studies:

Twelve varieties, differing in kernel type and maturation time, were provided by the seed companies Caussade, Pioneer and Codisem. Of the 12 cultivars, one (Fergus) is included in the Irish Recommended List 2012 suitable for growing in the open without plastic and Justina and PR39d60 are included in the Irish Recommended List 2012 suitable for growing covered/with plastic. The other 9 cultivars had all shown a high yield performance under Irish climate conditions. The varieties also differed in kernel type (flint, dent and flint-dent) and maturation time. The physiological experiments were to establish suitable temperature regimes for seed germination and root and shoot growth. Germination was classified as, when either the radicle or shoot emerged from the meristems, and these were at least 1 mm long. Three different light/dark temperature regimes were set up. A high temperature cycle was set at 22°C for 16 hours in the light and 18°C for 8 hours in the dark, a medium temperature cycle at 18°C for 16 hours in light and 12°C for 8 hours in dark, and a low temperature cycle at 10°C for 16 hours in light and 4°C for 8 hours in dark. Maize seeds were arranged on a surface of capillary matting lying over two layers of blotting paper to keep the environment moist without excess free water. The trays were placed in Snijder Microclima controlled environmental chambers at the three different temperature regimes described above. The layers of blotting papers and capillary matting were kept constantly moist by the addition of 100 ml of water every 24 hours. The individual seed trays were distributed in the cabinets according to a randomised block design and their arrangement was changed each time measurements were taken. Seed germination was determined every 24 hours. All experiments were carried out in triplicate. The goal of the experiment was to determine the germination rate and the growth performance of the genotypes, in terms of primary root and shoot elongation, under the cold treatment.

Samples from root and shoot tissues were taken for macro and micro array experiments. A custom cDNA macro array with known cold tolerance genes was constructed in UCD. A maize 46K array with 70-mer oligos was available from the University of Arizona (http://www.maizearray.org/index.shtml) which also supplied a hybridisation service. Four varieties were selected from the physiological experiment on the basis of the percentage of growth reduction, and used for the microarray analysis. One of the main objectives of the project was to identify expression patterns associated with temperature tolerance at the establishment stage in maize varieties adapted for NW European conditions. An understanding of such expression changes can provide tools for marker assisted breeding programmes. Analysis of variance was used to determine significant gene effects. Macroarray analysis was initially conducted, on cDNA of known maize cold resistance genes, in order to test if it was possible to detect changes, between the two experimental conditions, in the gene expression pattern of the varieties included in the Irish Recommended List, before the samples of interest were employed for microarray analysis. The macroarray analysis was performed by preparing a custom array consisting of ~300 cDNAs with a set of testing probes.

Four varieties selected from the physiological experiment on the basis of the percentage of growth reduction, were used for the microarray analysis. Roots were collected from seedlings grown under the same

Contact Susanne Barth Email: susanne.barth@teagasc.ie.

experimental conditions (18°C/16 hours for the control temperature regime and 12°C/8 hours for the low temperature regime, in dark conditions). Root samples were harvested at day 4, 5, 6, 7, and 8 days post-germination. At each time point, three biological replicates of root samples per variety were collected. The three biological replicates from day 4 were used for microarray analysis. An interwoven loop design (Kerr & Churchill, 2001) was applied to microarray analysis. The microarray analysis results showed that the two varieties with the highest degree of cold tolerance each had a set of differentially expressed genes (up and down regulated, p-value <0.05; variety 1, 39 out of 46K genes, variety 2, 29 out of 46K genes), while no genes appeared to be up or down regulated for the two cold sensitive varieties. Four of the differentially expressed genes were shared by 2 cold tolerant varieties, although not with the same degree of regulation. In particular, one of the genes was regulated in the opposite direction in two genotypes.
The outcomes of the microarray work were verified in a time series experiment with 5 time points (day 4 to day 8 post-germination). The gene expression was not maintained across the time series, but it was subjected to fluctuation. Nevertheless, except for one gene, the expression pattern was similar between the two varieties.

4. Main results:

Cold tolerance in maize root growth at early developmental stages is not dependant on the kernel type and maturing time, but varies according to the genotype and the time point at which measurements are made.
Four genes were significantly regulated under cold stress conditions in both cold tolerant varieties, but only three presented a similar expression pattern between the two genotypes, indicating a common mechanism for coping with the low temperatures.

5. Opportunity/Benefit:

Although directed specifically at maize the results will have applicability to other crop plants. The availability of detailed information on the maize genome will allow us to identify common genes/gene products that are a feature of plant responses to cold stress. An understanding of the genetic basis of plant response to low temperature is a key requirement if we are to increase the availability of crops/products suitable for temperate regions.

6. Dissemination

The project resulted in a number of scientific publications and presentations at meetings. The project was also presented to visitor groups and as a poster at the Tillage Conference in 2010.

Main publications:

Di Fenza M. (2013) Examining the physiological and genetic response of maize to low temperature conditions. PhD thesis, National University of Ireland.

Di Fenza M., Gallagher T.F., Hogg B. and Barth S. (2010) Examining the physiological and genetic response of maize to low temperature conditions. *Proceedings of the Irish Plant Scientists' Association Meeting* 2nd to 4th June 2010, UCD Dublin.

Compiled by: Dr. Susanne Barth

Contact Susanne Barth Email: susanne.barth@teagasc.ie.