AMARANTH:FUTURE-FOOD

Adding Value to Holy Grain: Providing the Key Tools for the Exploitation of Amaranth - the Protein-rich Grain of the Aztecs. Results from a Joint European - Latin American Research Project

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The project group in the amaranth field at San Luís Potosí, Mexico.

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Summary

The project AMARANTH:FUTURE-FOOD (www.amaranth-future-food.net) was a joint research project financed by the European Commission in the Specific International Cooperation Activities (INCO) of the 6th Framework Programme. The project group consisted of 11 partners from Mexico, Nicaragua, Argentina, the Czech Republic, Spain and Denmark. Amaranth is a protein-rich and gluten-free pseudo-cereal grain that was the basic food in South America and Mexico thousands of years ago. 60-70 *Amaranthus* species are known. The overall objective of the project was to provide the tools for an extensive and sustainable exploitation of amaranth. The project began on 1 September 2006 and ended on 31 December 2009.

A number of scientific papers with results from the project were published and more are under way. This publishable final activity report presents selected results from our research into the industrial use of amaranth and the use of amaranth as food, feed and food additives together with our results from extensive cultivation trials on 18 different amaranth genotypes in five research sites with varying climate. Results from our studies on drought and insect resistance and weed compatibility of amaranth genotypes are also described. In this report, we also share our experience with the audience on our end-user focus in two Nicaraguan women's agricultural cooperatives. The members of these cooperatives implemented amaranth cultivation and developed amaranth food products adapted to traditional Nicaraguan taste.

Table of contents

Contractors

Partner no.	Partner name and homepage	Partner short name	Country
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4	Centro de Investigación y de Estudios Avanzados del I.P.N. www.ira.cinvestav.mx	Cinvestav	Mexico
5	Crop Research Institute www.vurv.cz	CRI	Czech Republic
6	AMR AMARANTH a.s www.amaranth.cz	AMR AMARANTH	Czech Republic
7	Universidad Nacional Autónoma de Nicaragua – Managua www.unan.edu.ni	UNAN/ Managua	Nicaragua
8	Asociación Chinantlán, construyendo hermandad www.redoc.nl/chinantlan	CHINANTLÁN	Nicaragua
9	Universidad de Lleida www.udl.es	UdL	Spain
10	Centro de Investigación y Desarrollo en Criotecnología de Alimentos www.cidca.org.ar	CIDCA	Argentina
11	Universidad Nacional de La Pampa, Facultad de Agronomía www.agro.unlpam.edu.ar	UNLPam	Argentina

Overall objective of the project

The immediate objective of this project is to provide the tools for an extensive and sustainable exploitation of amaranth. The project will contribute to the overall development objective of providing health promoting food and exploiting the industrial use of amaranth and thus provide a source for income in regions of the world in which the warm and dry climate makes the cultivation of amaranth the obvious choice.

1. Project execution

1.1 Preparation of modified amaranth proteins as nutritional food ingredients

1.1.1 Objective

In order to improve the industrial exploitation of the amaranth proteins as a nutritional food ingredient our goal was to prepare modified amaranth protein isolates with improved functional properties and with health promoting effects.

To reach this objective we planned to isolate the proteins from amaranth flour and modify them by enzymatic hydrolysis to obtain peptides of different sizes. The different protein preparations would be tested with regard to their functional properties, foaming and emulsification and with regard to their antioxidant, anti-tumour and blood pressure regulatory activities.

1.1.2 Methodology

The composition of proteins of several amaranth varieties were analysed by electrophoresis. *Amaranthus hypochondriacus* and *A. mantegazzianus* were chosen to study their proteins because they showed outstanding differences.

Proteins were extracted with alkaline water, concentrated by precipitation and freeze-dried. Hydrolysates with a different degree of hydrolysis were obtained by treating the proteins at different times with two enzymes, trypsin and alcalase. The structure and physicochemical properties of all samples were analysed by electrophoresis, calorimetry, ultraviolet and fluorescence spectroscopy and chromatography. Fractionation of peptides was carried out by chromatography and electrophoresis.

The surface properties of the samples were determined by measuring the air-water surface tension decrease, the dynamic interfacial tension and the interfacial rheology in oil-water interface, where the water face was a protein solution. Foaming and emulsifying properties were studied analysing the capacity of formation and stabilisation of foams and emulsions made with the sample solutions.

Antioxidant activity was studied by testing the capacity to inhibit the linoleic acid oxidation and to neutralise $ABTS^{+}$ free radical.

In vitro antihypertensive activity was studied by measuring the capacity to inhibit the blood

pressure regulatory enzyme ACE, and *in silico* studies were carried out on the reported sequence of the cDNA of an amaranth globulin (http://www.ncbi.nlm.nih.gov/sites/entrez).

The potential anti-tumour activity was studied analysing the capacity to inhibit a carcinogenic cell line (UMR106) proliferation.

1.1.3 End results

According to the structure of the sample proteins, trypsin (TH) and alcalase (AH1.7; TH 9.5) hydrolysates with a low degree of hydrolysis were chosen to study their functional properties (Condés *et al.,* 2009). They contain small peptides and low size proteins keeping some structure. On the other hand alcalase hydrolysates with a high degree of hydrolysis (AH30), formed by free, small peptides were chosen to study their biological activities. The hydrolysates' behaviour was compared with the non-treated proteins (AI).

All proteins presented higher solubility at acid and alkaline conditions but hydrolysates proved to be more soluble than non-treated proteins at neutrality (Condés *et al.,* 2009). Considering the pH of foods and solubilities, functional properties were studied at very acid (pH 2), neutral and mild alkaline media.

It was found that in the acid medium the non-treated and heat-treated proteins and trypsin hydrolysate were the best ingredients for food foams, whereas at around neutrality the heat-treated proteins are the recommended additive for those foodstuffs (Figure 1.1.1).

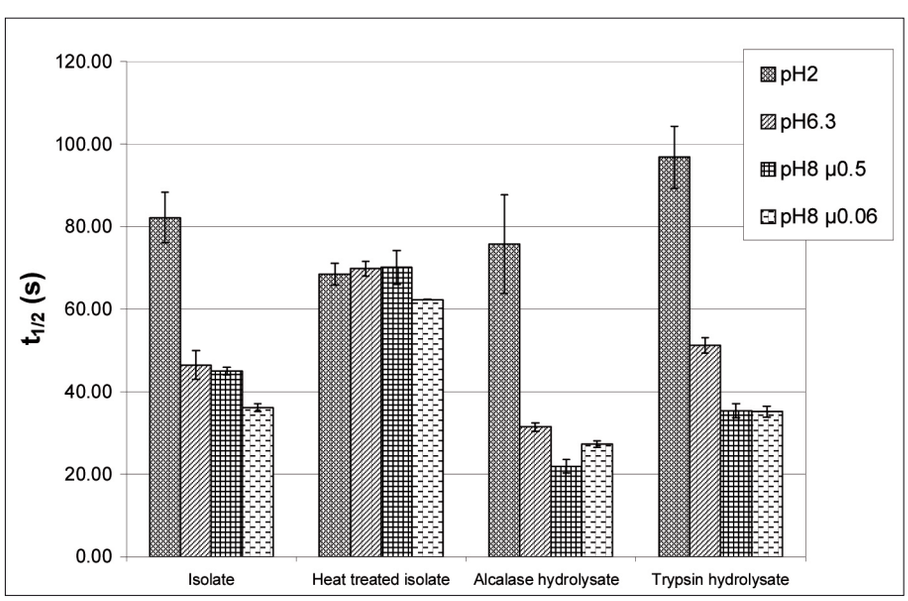

Figure 1.1.1. Stability of foams as the time of half-drainage of the incorporated liquid ($t_{½}$).

In a similar way the acid medium was the best condition for the studied samples to develop good emulsions and the best ingredients were the non treated proteins and the trypsin hydrolysate (Figure 1.1.2) (Ventureira *et al.,* 2010). Considering that in the acid medium proteins are partially unfolded, these results suggest that the best structural characteristics for proteins to develop good foaming and emulsifying properties are large, partially unfolded molecules.

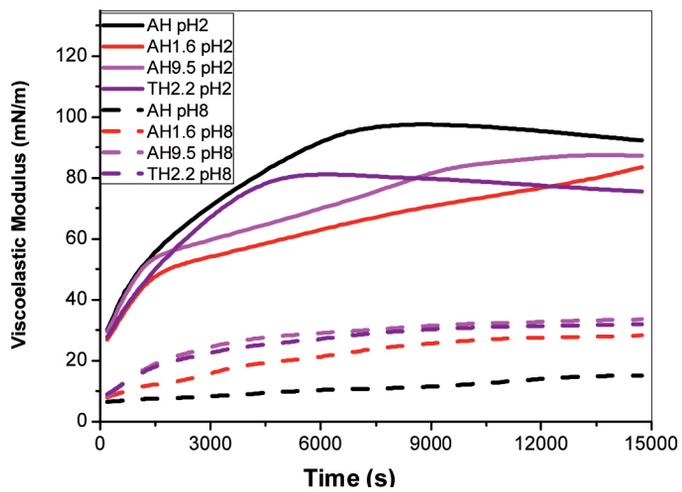

Figure 1.1.2. Interfacial rheology vs time of o/w interface of the samples (samples and pH are indicated in the figure).

In vitro antihypertensive, antioxidant and antitumoural activities were found in amaranth proteins.

By means of theoretical studies two small peptides encrypted in an amaranth globulin sequence were predicted to present antihypertensive activity, these results were validated by *in vitro* experiments (Figure 1.1.3) (Vecci and Añon, 2009). The alcalase hydrolysate also presented antihypertensive activity and its smaller peptides fraction showed the highest activity. The antihypertensive activity of the hydrolysate was also demonstrated by *in vivo* experiments using hypertensive rats (Fritz *et al.,* 2010).

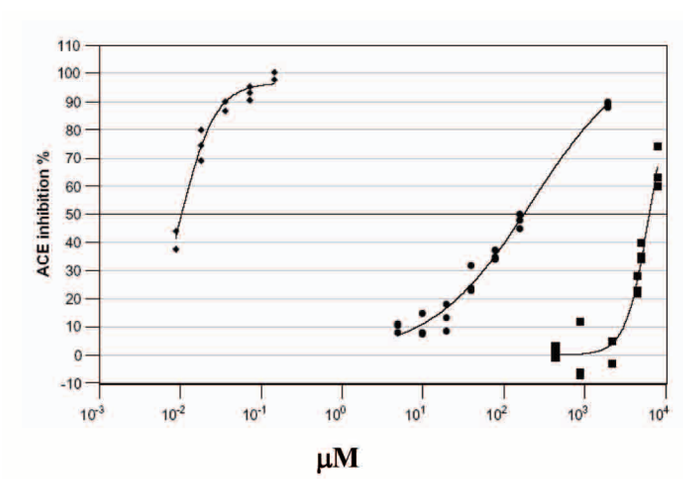

Figure 1.1.3. Ace inhibition curves of captopril (♦), VIKP (●) and ALEP (■). Continuous lines represent the non-linear regression using Hill's equation for each data set.

Non-treated proteins and the hydrolysate showed antioxidant activity. Several peptide fractions were isolated that developed antioxidant capacity by different mechanisms (Figure 1.1.4)

(Tironi and Añon, 2009). Non-treated proteins and the hydrolysate showed a higher antioxidant activity after simulated gastrointestinal digestion.

Concerning the potential anti-tumour activity results showed that the non-treated proteins presented this property, which was enhanced after the hydrolysis (Barrio and Añon, 2010). Small peptides were isolated that showed the highest *in vitro* anti-tumour activity (Tables 1.1.1 and 1.1.2). It was found that proteins developed this capacity by inhibiting cell adhesion from a tumour cell line, inducing necrosis and apoptosis (Figure 1.1.5).

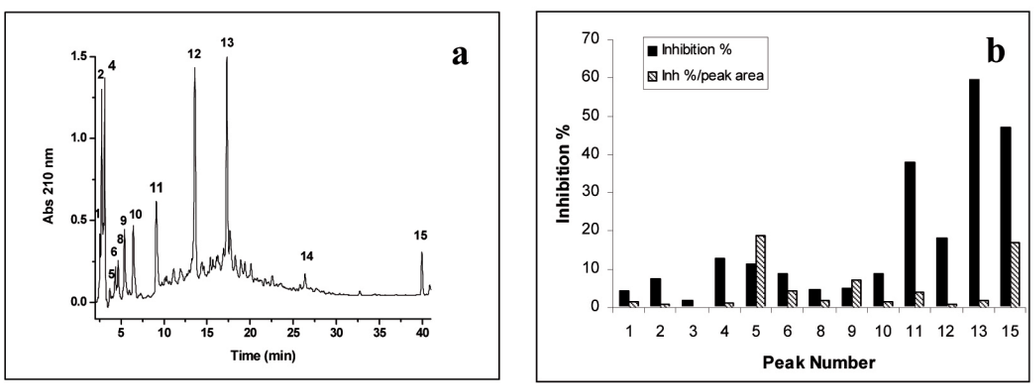

Figure 1.1.4. (a) RP-HPLC preparative chromatogram of **AH** indicating the peak fractions; (b) $ABTS^{+\cdot}$ scavenging activity of peak fractions represented as Inhibition % and Inhibition %/peak area.

Table 1.1.1. Inhibition of UMR106 cells proliferation.

Fraction	**IC_{50} (mg/ml)[(a)]**
AI	1,0
Albumin	0,5
7S-globulin	> 2,0
Glutelins	> 2,0
DMSO-pep[(b)]	0,1

[(a)]IC_{50} concentration of protein that decrease 50% cell proliferation. [(b)]Amaranth peptides preparation.

Table 1.1.2. Inhibition of UMR106 cells proliferation by HPLC fractions of DMSO-pep[(b)].

HPLC peak	**IC_{50} (mg/ml)[(a)]**
1st	0,06
2nd	0,1
3rd	0,05
4th	0,04
5th	0,02

[(a)]IC_{50} concentration of protein that decrease 50% cell proliferation. [(b)]Amaranth peptides preparation.

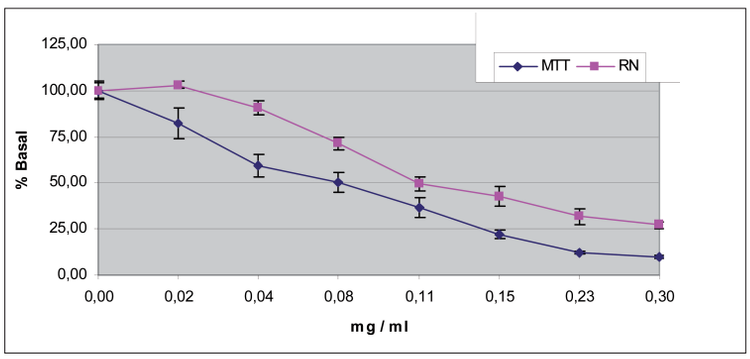

Figure 1.1.5. DMSO-pep cytotoxicity on mitochondrial (MTT) and lysosomal (RN) function.

1.1.4 Impact of the project

In conclusion, the results of this work described the preparation of three amaranth protein ingredients, non-treated, heat-treated and trypsin-hydrolysed proteins, with acceptable functional properties. The best conditions for developing solubility, emulsifying and foaming capacities were determined.

Health promoting effects of amaranth proteins were determined, and the production and isolation of amaranth peptide fractions with potential biological activities were described.

Though the investigations on the area are not going to stop at this point, the objectives have been reached.

This work results bring new knowledge about the relationship between protein structure and foaming and emulsifying functional properties. This information, which was reported at conferences and in journals, is a contribution to the food science field that is the basement of technological developments.

The physiological activities, antioxidant, potential anti-hyperthensive and antitumour, of amaranth peptides were shown at a molecular level, which represents an important contribution to the new science of functional foods.

The preparation of amaranth proteins with improved functional properties and physiological effects will encourage the industry to use amaranth as a source of excellent protein ingredients. This information was reported in international journals and shown to members of the industry at meetings and conferences.

The spreading of new knowledge about the health promoting effects of amaranth proteins will stimulate the industrial production of bioactive ingredients making possible the increase of the internal and international market.

During the course of the investigations some students were interested in the amaranth protein properties. This promoted the development of final works (undergraduate thesis) and the incorporation of new scholars to search for proteins with new physiological activities (anti-clotting).

1.2 Metabolic profiling, identification and isolation of phytochemicals and glutelin peptides with health effects

1.2.1 Objective

The objectives of this research are to isolate and identify secondary metabolites from amaranth grain, plants and flowers, to assess the variation in phytochemical content of amaranth grains and leaves from different varieties, to study the antioxidant and anticancerogenous activities of the phytochemicals present in major concentrations and to characterise the antihypertensive function of amaranth tryptic-digested glutelin peptides.

1.2.2 Methodology

A good and efficient procedure was developed for isolating secondary metabolites from amaranth leaf samples. The procedure is initiated by methanolic extraction at room temperature of the freeze-dried plant material. Afterwards lipids are removed by washing with heptan and the rest of the extract is separated into six fractions of decreasing polarity by solid face extraction (SPE) on C-18 column material. These fractions are then called polarity fractions. The relevant fractions are then separated initially by medium pressure liquid chromatography (MPLC) chosen for the large loading capacity: the resulting fractions are separated by repeated high performance liquid chromatography (HPLC) chosen for the high separation performance (Steffensen *et al.,* 2010c). The solvent systems for the liquid chromatography must be optimised for each fraction.

A quantitative LCMSMS analytical method for the analysis of phenolic acids and flavonoids in amaranth grains was developed and validated (Barba de la Rosa *et al.,* 2009). Similarly, a quantitative analytical method for the analysis of phenolic acids, flavonoids, cinnamoylamides, trigonellin and betain in amaranth leaves was developed (Steffensen *et al.,* 2010b).

The antihypertensive function of amaranth glutelin peptides was analysed by the angiotensin converting enzyme (ACE) inhibitor using the spectropohotemetric assay and the hippuryl-histydil-leucine peptide. *In vitro* vasodilatiation activity was measured in rat aortic rings.

1.2.3 End results

Isolation and identification

Trigonelline and betaine were identified as major constituents of the most hydrophilic polarity fraction (water extract). These compounds are known to relate to salinity tolerance and drought stress in plants. The flavonoids rutin, isoquercitrin and nicotiflorin were similarly identified as major constituents of the methanolic extract. N-Feruloyl-4-*O*-methyldopamin and *N*-feruloyltyramin were isolated from the methanolic extracts in both a cis and trans isomer (Steffensen *et al.,* 2010b; Pedersen *et al.,* 2010).

The six polarity fractions were subjected to proliferation assays and cytotoxicity assays in order to determine if the fraction exhibited any activity upon the cells. The four first polarity fractions showed no toxicity towards the cells, the fifth showed some activity, but the most potent activity was exhibited by the sixth polarity fraction. These results encouraged us to try to identify some constituents of this fraction. The fraction was repeatedly chromatographed using preparative

HPLC-DAD and analysed by NMR. The attempts failed, however. No compound has so far been obtained in an amount sufficient for NMR identification.

The hydroxycinnamic acid amides (HCAA) *N*-feruloyl-4-*O*-methyldopamin and *N*-feruloyltyramin, isolated from the amaranth, belong to a group of compounds common in many plants that play a role in the defence mechanisms of the plants. For this reason and for the relative simplicity of the synthetic routes we initiated the synthesis of a library of HCAAs. Some have previously been detected in different plants, while others still remain to be identified from natural sources.

Nine of these HCAAs were selected and produced in sufficient amount for currently ongoing biological tests. The entire library was also used for screening of amaranth leaves of different species. It was shown that 6 of the HCAAs were present in the leaves of amaranth. All the HCAAs identified by this screening are known from natural sources but only one of these has previously been reported from amaranth. The structure of the remaining 5 HCCAs is published in Pedersen *et al.* (2010).

In general the isolation and identification of standards from amaranth has been more time-consuming than anticipated. This was counteracted by the synthesis of the HCAA library. The synthesis of standards, though, is only feasible in special cases where starting materials are commercially available and the steps of synthesis are simple and few.

Quantitative metabolic profiling

The content of the flavonoids rutin and nicotiflorin varied between 4.0 and 10.2 μg/g and 7.2 and 4.8 μg/g respectively, in amaranth seed flour from two commercial (*Tulyehualco* and *Nutrisol*) and two new (*DGETA* and *Gabriela*) varieties of *A. hypochondriacus* grown in the Mexican Highlands zone. A preliminary screening of 33 flavonoid compounds showed that only rutin, nicotiflorin and isoquercitrin were present in amaranth seeds from 18 field tested genotypes, grown in Mexico, Spain, the Czech Republic and Argentina and amaranth leaves from two field-tested genotypes (Steffensen *et al.,* 2010a; 2010b).

The quantitative LCMSMS method for analysis of flavonoids and phenolic acid were used for analysis of the above-mentioned seed samples. Generally, rutin and nicotiflorin are the most abundant secondary metabolites of those analysed for in the amaranth seeds. Isoquercitrin was only present in some samples and in very low concentrations. The flavonoid concentrations in seeds are seen in Figure 1.2.1. Figures 1.2.2 and 1.2.3 show the content of the phenolic acids in the seeds. Protocatechuic acid is generally the most abundant of the phenolic acids. No obvious patterns can be seen in the quantification data. Principal component analysis (PCA) was applied to detect patterns that relate to different aspects of the trials. Conclusions on the PCA data analysis will be revealed in Steffensen *et al.* (2010a).

Two genotypes of amaranth leafy vegetable were cultivated in the field trials in Spain, Argentina and the Czech Republic. The quantitative analytical method was amplified for analysis of flavonoids, phenolic acids, cinnamoylamides, trigonellin and betain. The concentrations of the flavonoids and the phenolic acids are presented in Figure 1.2.4 and Figure1.2.5, respectively. The

concentration levels of cinnamoylamides, trigonellin and betain will be revealed in Steffensen *et al.* (2010b).

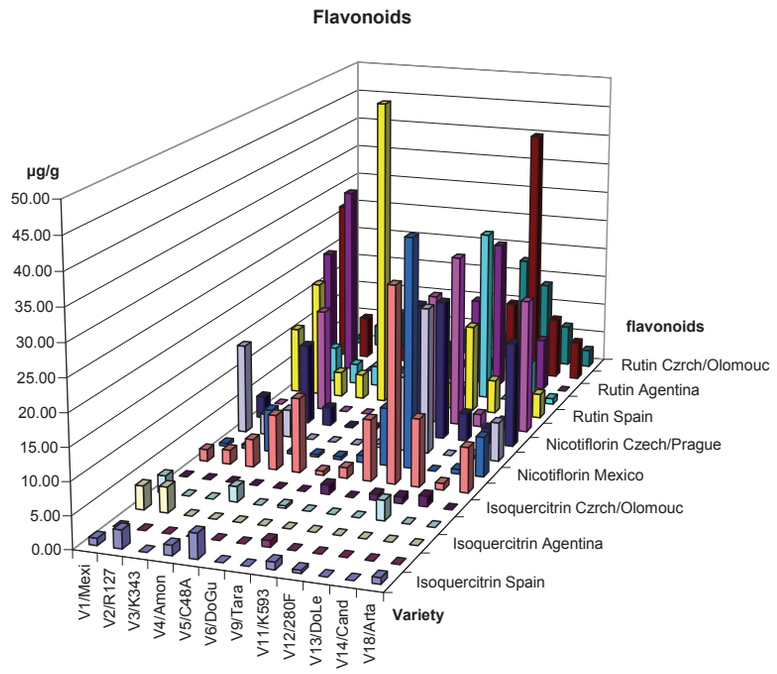

Figure 1.2.1. The content of the three flavonoids, rutin, nicotiflorin and isoquercitrin in 12 *Amaranthus* genotypes. Only the results from the genotypes that were able to grow at all 5 trial locations are presented in this figure.

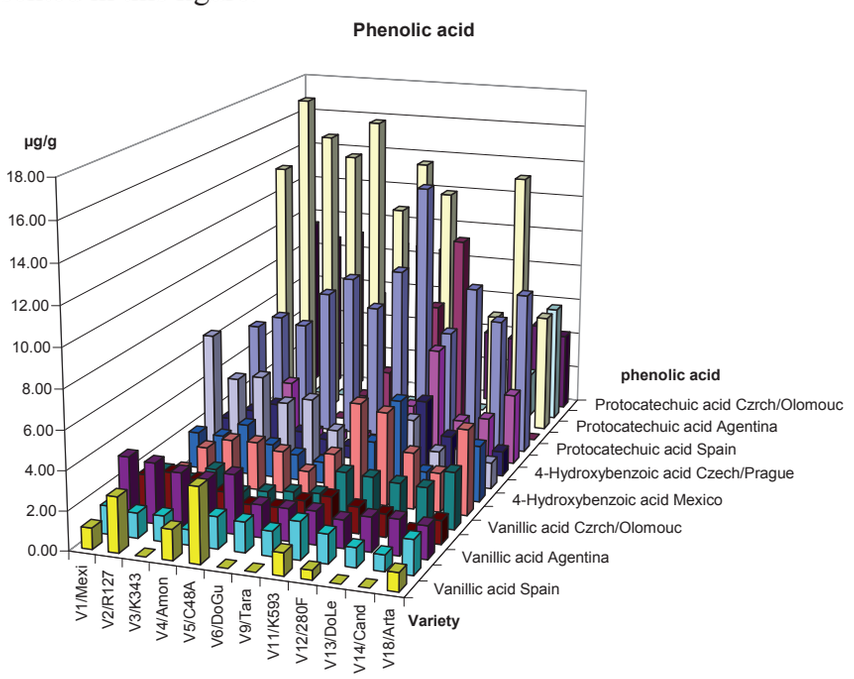

Figure 1.2.2. The content of the phenolic acids, protocatachuic acid, 4-hydroxybenzoic acid and vanillic acid. Only the results from the genotypes that were able to grow at all 5 trial locations are presented in this figure.

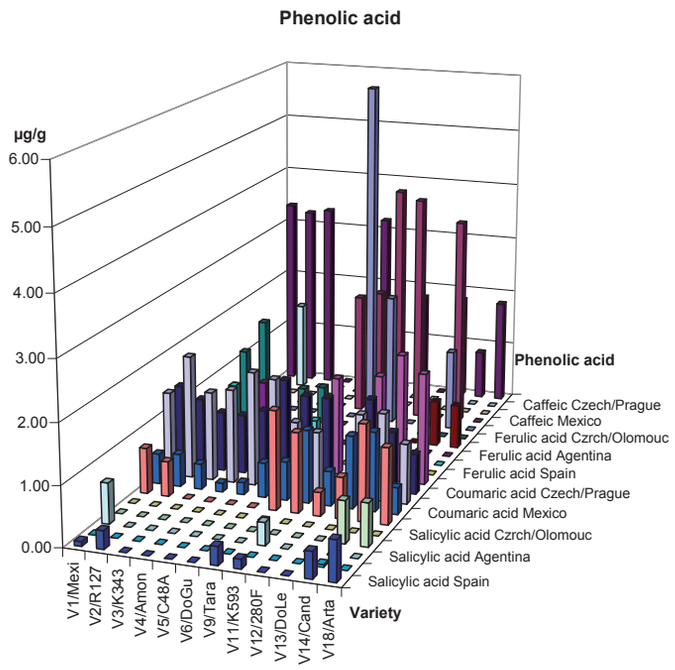

Figure 1.2.3. The content of the phenolic acids, caffeic acid, ferulic acid, coumaric acid and salicylic acid. Only the results from the genotypes that were able to grow at all 5 trial locations are presented in this figure.

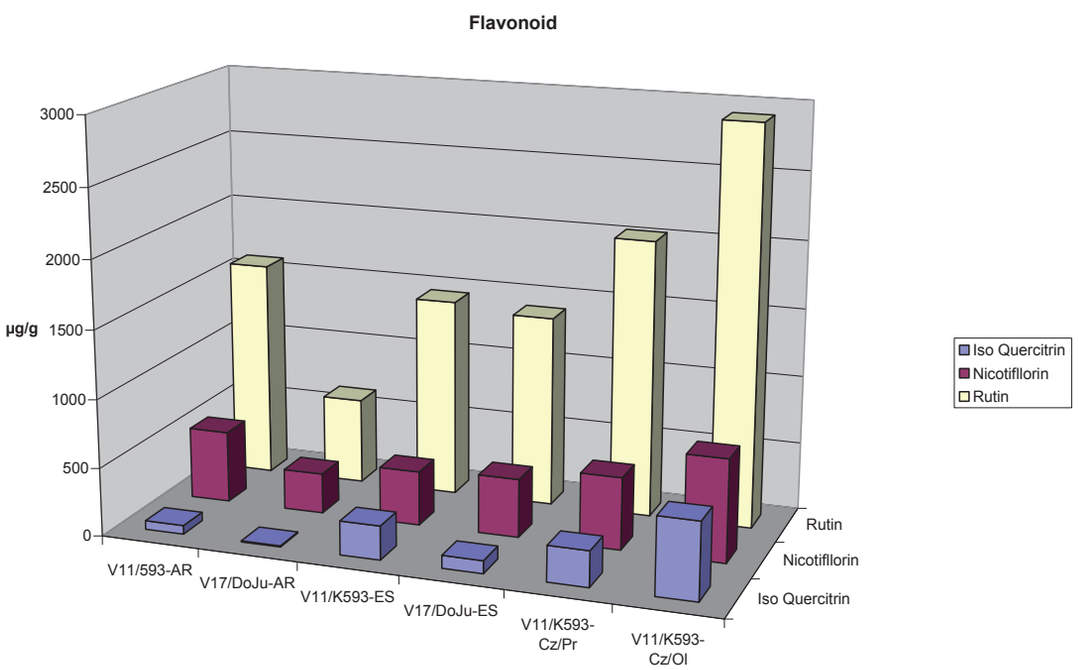

Figure 1.2.4. Content of the flavonoids in two genotypes of leafy vegetable amaranth cultivated in Argentina, Spain, the Czech Republic, Prague and the Czech Republic, Olomouc.

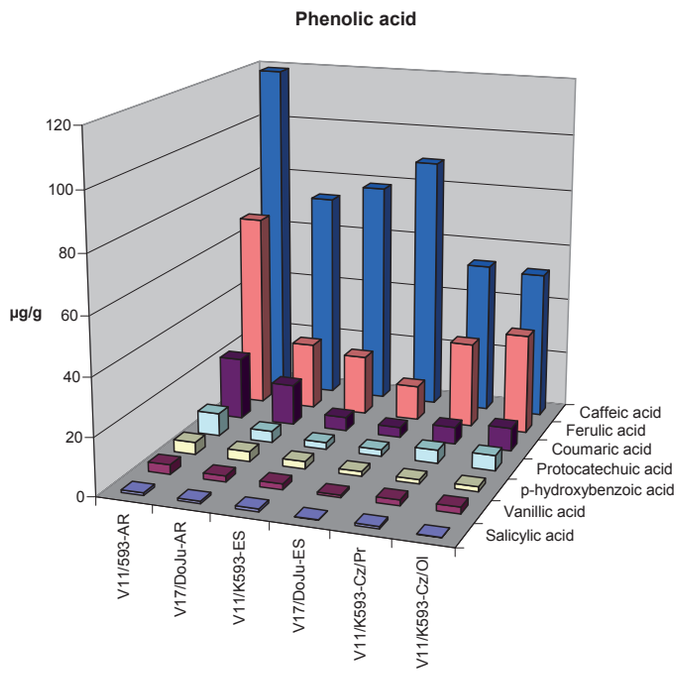

Figure 1.2.5. Content of the phenolic acids above detection limit in the two genotypes of leafy vegetable amaranth cultivated in Argentina, Spain, the Czech Republic, Prague and the Czech Republic, Olomouc.

Biological activity of phytochemicals

A number of the isolated compounds, e.g. rutin, betain, trigonellin, 3,4-dihydroxybenzoic acid, selected hydroxycinnamic acid amides were analysed for their cytotoxic activity. Cytotoxic activity from any of the compounds analysed until now was only seen when extreme concentrations were tested. We therefore conclude that a concentration of these compounds like those found in the seeds and leaves are of no risk to the health of the consumers (Steffensen *et al.,* 2010d).

Antihypertensive activity of glutelin peptides

From previous reports of LC-MS/MS analysis of the amaranth glutelin fraction digested with trypsin 508 new peptides were reported. The profile of peptide activity showed that the main functions were antihypertensive, ubiquitin-mediated proteolysis, antithrombotic, antiamnestic, opioid, antioxidant and as a neuropeptide among others (Silva-Sánchez *et al.,* 2008). Based on these results we decided to further characterise the antihypertensive activity of glutelin peptides. The vasodilatory effects of amaranth glutelin digests were tested by using rat aortic rings. Our results show that peptide glutelins induced vasodilation comparable with Ach (Figure 1.2.6). We also demonstrated that vasodialtory effects were completely blocked by a pretreatment with HOE-140, suggesting an important role of the BK (Barba de la Rosa *et al.*, 2010).

1.2.4 Impact of the project

The presence of biologically active secondary metabolites (phytochemicals) in agricultural crops is important for several reasons. Such compounds often have health promoting effects,

but can be anti-nutrients as well. Biologically active secondary metabolites often enhance the resistance of crops towards weeds, insects and diseases. Very limited knowledge on the variation in the content of secondary metabolites in amaranth seeds and leaves existed, when we initiated our project. The main efforts until then according to the scientific literature focused on betalanins and amaranthins that give the red colour to amaranth leaves. We showed that high concentrations of several flavonoids with anti-oxidant activity are present in both seeds and leaves, which is of importance to the consumer of amaranth seeds and leaves. We expect that our discovery of a range of biologically active hydroxycinnamic acid amides and a rare terpene, a rare phenolic compound and two saponins in amaranth leaves can be used for future focus on health promoting effects for the consumer and for defence properties of the amaranth crop in sustainable agriculture. Natural biopeptides have low potency as bioactive compounds, but because they are regularly ingested in the diet, their long-term physiological effects are perceptible. We have demonstrated that amaranth seeds are a natural source of nutraceutical compounds such as biopeptides and phytochemicas compounds. This knowledge will increase their importance as a potential source of potent antioxidants in the human diet.

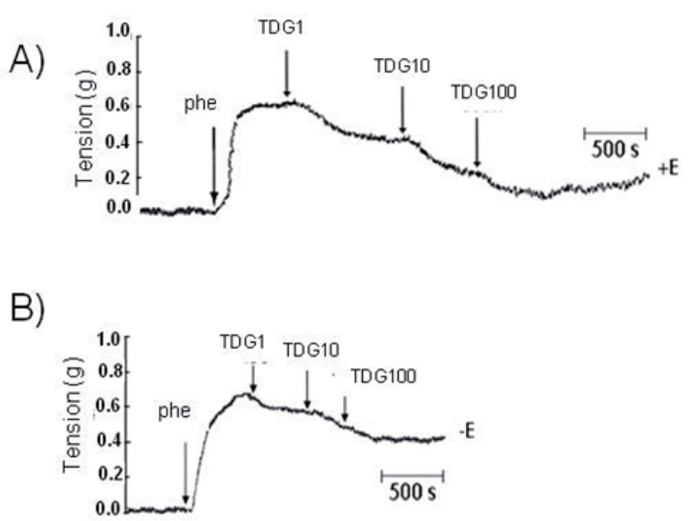

Figure 1.2.6. Tryptic-digested glutelin (TDG) peptides induce endothelium-mediated relaxation of rat aortic rings. Vessels were precontracted with 50 nM phenylephrine (Phe) and treated wit increased concentrations (1-100 µg/ml) of A) TDG in the presence of endothelium, B) TDG in the absence of endothelium.

1.3 Use of amaranth for production of amaranth oil containing squalene, as energy crop, as food and as feed

1.3.1 Objectives

The main goal of the energy task was to prove or disprove the suitability of the amaranth plant for energy purposes. The growth of amaranth green biomass was studied. A detailed chemical analysis was made, and the quality of thermal conversion and anaerobic fermentation (production of biogas) was studied. The ash deformation temperature and the net calorific value were calculated. New possibilities of processing amaranth plants were studied within task "Food". The use of young amaranth leaves, amaranth sprouts and amaranth inflorescence was evaluated. A technique for continuous harvest of young amaranth leaves in greenhouses was evaluated, and changes in the nutritional level of growing amaranth leaves were measured. Different ways of germination were applied and evaluated. New possibilities of utilising the amaranth inflorescence, e.g. as amaranth inflorescence tea, were drafted and then introduced to the market. The task concerning possibilities of using amaranth as feed looked at the prohibition of meat bone-meal (MBM) in Europe, which has set up the need to find a suitable protein feed. Amaranth grain or other parts of amaranth were studied as feed for pigs, poultry and carps. The growth effect on animals was measured. An overall conclusion including economic aspects was made. The goals of the "Oil" task consisted of a description of the best method of obtaining amaranth oil from amaranth grain, determination of the significant nutrients present in amaranth oil and development of a formulation of an improved product based on amaranth oil. It was necessary to concentrate on the implementation of the chromatographic analytical techniques required to determine the squalene content in the extracted oil.

1.3.2 Methodology

Amaranth plants were harvested at two localities in the Czech Republic: in Prague and in Olomouc. Different genotypes were planted. The chemical analysis and quality of thermal conversion were made in a specialised laboratory. The anaerobic fermentation of amaranth and the production of biogas were studied by our company in a Bioreactor Bio stat B.Braun Bitech. Practical tests of the production of leafy amaranth were done in greenhouses. These samples were observed at various stages of their growth, and further laboratory monitoring was done. Available information regarding the sprouting of amaranth grain was gathered. Germination on clay plates, in Eschenfelder bottles and in Climacell 404 machine was tested.

Pigs: Part of the experiments with pigs, which were done by AMR Amaranth a.s. in Veterinary Research Institute in the Czech Republic, studied the growth efficiency effect of feeding amaranth to pigs; meanwhile the other part was concerned with the health results in comparison with the control meat and bone meal (MBM) diet. Pigs were divided into three experimental groups fed dried amaranth and one already mentioned control group. Experimental pigs were fed dried above-ground amaranth biomass, amaranth grain heat-treated by popping and non-heat-treated amaranth grain.

Poultry: Tests with the same focus as the above-mentioned pig tests were done with poultry. Research on the growth efficiency and health aspects of poultry was performed with non-heat-treated amaranth meal, heat-treated amaranth meal and meat-and-bone meal.

Carps: An experiment with amaranth for carp feed was done. Three groups of fish were tested - young carps, which were fed primed amaranth seeds, and two control groups, one fed

according to standard procedures used in fish farming and the other receiving no specific diet. The amaranth seeds were put into the water for two days. All three groups of carps were kept in controlled conditions and compared.

Methods for obtaining amaranth oil from milled amaranth grain were described: extraction with different organic solvents (hexane, heptane), cold pressing (temperature lower than 60°C and supercritical extraction CO_2). Isolation and purification procedures were developed with the use of supercritical carbon dioxide extraction followed by analysis in gas chromatographic equipment and HPLC equipment (Perkin Elmer).

1.3.3 End results

One amaranth plant can produce up to 400 leaves, and the stem diameter can be up to 40 mm. These outputs suggest that amaranth biomass has big yields and is thus potentially very attractive for energetic purposes. The chemical analysis showed that amaranth could possibly become an energy crop although its natural ability to cumulate toxic compounds from soil should be taken into consideration. These toxic compounds can react further during combustion. The high heating value of amaranth is 4.4 MJ/kg and the low heating value is 4.03 MJ/kg (for comparison HHV of cereals is 5.69 MJ/kg and of brown coal 28 MJ/kg). In a Bioreactor Bio stat B.Braun Bitech amaranth was concluded to be a low-grade fuel in comparison with coal; however, as a biogas it is of medium quality. Its maximum of producing biogas is at 0.35 l/g of dry content at the 40th day of an experiment.

Most of the amaranth species have edible young leaves, but as a vegetable are mainly used the relatives of *Amaranthus tricolor*. The greenhouse results showed that young leaves of amaranth contain more than 25% protein (compared to 11-12% of protein in the grain). The mineral content increases till the 8th week of growth and potassium till the 10th week. In order to have a high content of protein and oil the first harvest of young amaranth leaves should be timed to the 6th week of maturity. It is possible to harvest amaranth leaves for protein content four times per season in greenhouses.

In laboratory conditions the best sprouts were germinated in Eschenfelder bottles. On clay plates it took very long to get enough moisture, and Climacell 404 was not as effective as we expected because of the costs of energy.

Amaranth cultivated for inflorescence should be harvested after approx. 60 days of growth. The inflorescence has a high content of bioflavonoides such as rutin, quercetine and others. A new product, Amaranth tea, was invented and introduced into the market. This tea has a beneficial effect on stomach pain and dysmenorea. Another utilisation is the extract based on the inflorescence with the concentrated content of bioflavonoides - mostly rutin. This extract is done by water extraction, and it must be stabilised by alcohol, fructose or propylene-glycol.

Pigs: The conclusion of the measurements are very optimistic: not only were there no significant difference in the body weight gain of amaranth-fed pigs in comparison with MBM-fed pigs, but the chemical content of amaranth, namely lipids and fatty acids like linoleic acid and squalene, could probably modify the fatty acid composition of pigs. The underskin store fat layer was thinner in the experimental group, and the taste of the meat was better. The highest

daily body gain was discovered in animals that were fed with the mixture containing heat-treated amaranth (pop amaranth) and the best conversion was detected in the group that was fed non-heat-treated grain.
Poultry: As for pigs, the groups of chickens fed amaranth obtained comparable results in all characteristics with the control group whose diet included a component of animal origin. Also, this experiment showed that there were no differences in weight gained between poultry fed heat-treated amaranth and poultry fed non-heat-treated amaranth. This is surprising since non-heat-treated amaranth contains antinutrients, like other raw cereals, that partly block the intake of zinc or iron. That could be caused by a relatively small portion (7%) of raw amaranth in the poultry's diet.
Carps: The measurements are very positive to amaranth. No antinutritional effect was obtained in the experimental group fed raw amaranth. By contrast, amaranth caused the largest growth in all three groups.

A SWOT analysis was done to evaluate amaranth as feed. As a result we recommend amaranth as a feed for its nutritional qualities. At the moment the greatest weakness is the absence of knowledge about amaranth within farmer communities.

The results of the "Oil" task also show very good perspectives – the perspectives for utilisation of amaranth oil is huge – from the pharmaceutical industry via the food and feed industry to the cosmetic industry. The results showed the unique composition of amaranth oil, whose most important nutrient is squalene. It can be separated from oil by extraction process, but it is not necessary, and oil works in different ways as a whole. Different methods of extracting amaranth oil from milled grain were compared, and the best method was found. The yield and economical perspectives were described.

1.3.4 Impact of the project

The results showed great opportunities of using amaranth as a plant for grain and leaf production for different industries. There are potential huge market perspectives in each tested industry. The composition of amaranth oil with the most important nutrient, squalene, should be sold as a nutrient raw material to the pharmaceutical industry (in vegetarian capsules), also to the cosmetic industry for different products for hair and body cosmetics. Extraction of squalene from amaranth oil is costly, and it is not necessary; amaranth oil works well in consistence as a whole. Amaranth plants should be grown for leaves for consumption and also as feed for animals. There is a possibility to use it also for the energy industry, but it shows more potential as a source of food and feed for humans and animals, respectively, than to combust it as a source of power. The presented results showed a good point for future investigations for improvement of amaranth as an available crop with commercial potential.

1.4 Amaranth cultivation in Mexico, Argentina, the Czech Republic and Spain

1.4.1 Objective

The main aims were to test different amaranth species in field trials. The selected genotypes of amaranth were cultivated in a randomised block design with three replicates in the Czech Republic, Mexico, and Spain and four replicates in Argentina. The main agronomic traits as well as pests, diseases and weeds from field trials in all countries were monitored, collected and photographed. The last objective was to estimate the cost and gross margin of amaranth production.

1.4.2 Methodology

The trials were performed in 2007-2008 in four different countries in five places (Argentina, the Czech Republic – Prague and Olomouc, Mexico, Spain). At all sites, meteorological data such as maximum, minimum and mean temperatures, relative humidity, precipitation and mean wind velocity were recorded during the trials. In addition, soil analyses including total exchangeable cationic capacity, organic matter contents, nitrates, ammonia, total nitrogen, phosphorus, potassium, calcium and magnesium and soil field capacity and silt, sand and clay composition were determined before planting. For the experiment, 18 genotypes (Table 1.4.1) selected by participants P4, P5 and P11 were grown in all sites, during year 1 while in year 2 some genotypes were excluded due to inferior performance.

The experimental plots consisted of six rows 15 m long and with 0.25 m between rows. The two external rows (i.e. the first and the sixth) were discarded as parcel borders. The first and last 0.50 m of each row was also discarded as parcel borders. The second row was used to take samples for analyses and to mark the ten reference plants for follow-up evaluation. The third, fourth and fifth rows were used for determining the field yield-related quantities. In the second row, 10 reference plants were marked. The sowing amount was 3 kg per hectare. Normal cultivation, fertilisation, irrigation and plant protection were applied according to necessity and the local cultivation practices used in each country. Harvest was done by hand. During the vegetative period, the following traits were evaluated on reference plants: stem diameter, number of leaves in four different development stages, harvest index, height at harvest, proportion of dark seeds, seeds' predominant colour, inflorescence length, weight of 1000 seeds, volume weight. In addition, selected phenological traits were recorded such as number of days from sowing to seedlings emergence, number of days from sowing until the leaves covered the inter-row space, number of days from sowing to beginning of anthesis and number of days from sowing to harvest. In harvest time, final plant population, parcel grain yield and seed moisture were also determined.

Other tasks were the monitoring, collection and photographing of main pests, diseases and weeds.

1.4.3 End results

Cultivation of selected amaranth genotypes

In the Czech Republic, sowing took place at two different times and at two different sites. In Prague seeds were sown in the middle of May and in Olomouc at the beginning of June. All characteristics were evaluated according to the technical report for field trials and according to

individual development stages. From the results is clear, that there were differences between the sites in the Czech Republic. In Olomouc the vegetative period was determined to be about 10 days shorter on average. The higher value of seed density and 1000-seed weight was also found in Olomouc. In contrast, the higher grain yield was in Prague. Due to their long vegetative period and early frost in the October 2007 the genotypes number 7, 8, 10, 15, 16 and 17 were excluded from the Czech field trials. In 2008 only 12 genotypes were evaluated. Genotype 12 was the most early-ripening in both places. In Prague it was harvested on average 100 days after sowing and in Olomouc 86 days after sowing.

Field trial results in Argentina showed also substantial differences in growing cycles between genotypes: those with the longest cycle were 7, 10, 13, 15, 16 and 17 (7 and 15 had to be excluded for further trials because they did not complete the cycle in Argentina), intermediate ones were 1, 2, 3, 4, 5, 11 and 18 and the shortest one was genotype 12. All genotypes, except No. 12 showed good defoliation at harvest. Thus genotype 12 was considered not apt for mechanical harvest, unless a chemical dessicant was applied, while genotype 18 showed the most promising aptitude for mechanical harvest, due to reduced plant height and good defoliation. Genotypes 9, 11 and 18 produced the highest yields in Argentina. In summary, both genotypes 11 and 18 were considered best choices for mechanical cultivation and harvest in Argentina.

In the trials carried out in Spain it was observed that varieties develop in cycles of varying duration. Variety 12 is the earlier with 90 days in its cycle length, while this is approximately doubled for varieties 15 and 16. This fact leads us to believe in amaranth as a suitable crop for rotation with other crops if the proper sowing date is adapted for each biotype. It is necessary to adapt the crop cycle in order to obtain an adequate size to allow it to be harvested with a combine machine (Zamora *et al.*, 2008).

Amaranth is a crop with lower requirements than maize as regards water and fertilisation. Optimum sowing density should be between 400,000 and 600,000 plants/ha with a separation between rows of 20 to 30 cm. The crop does not respond significantly to a greater amount of irrigation water, although it has critical needs in concrete moments such as emergence and, from literature, in the flowering period.

In either test period volunteer crops were observed in the next cropping cycle as a result of the low seed dormancy, its short soil longevity and its sensitivity to winter cold.

Two field trials were performed in Mexico in years 2007 and 2008. The third field experiment, in 2009, was terminated due to very low germination rates caused by unknown factors. Six additional genotypes to those listed in Table 1.4.1 were included in the above field trials. They included lines that are commercially cultivated in the central highlands of Mexico and others, originally from Nepal, that are believed to have non-shattering properties, which are a desirable trait when harvesting of the crop by mechanical means is contemplated. The results were very variable from year to year, suggesting that amaranth cultivation can be very sensitive to variations in handling during sowing and cultivation (e.g. thinning of plants was a crucial step, since it tended to drastically reduce plant growth if not done in time), soil quality and weather. In general, the Mexican genotypes, particularly genotypes 7 and 9, produced the best perfor-

mance in terms of yield and harvest index. The *A. cruentus* genotypes from Argentina and the Czech Republic produced good results as well, except for genotype 14 which produced very low yields even though the plants were vigorous and formed big seed heads. Some Mexican genotypes identified with different names (e.g. Rosita, Tehuacán, Morelos and Tulyehualco) produced phenotypes that were almost indistinguishable from each other in the field, This led us to believe that they correspond to the same genotype, which was not an unexpected event considering the lack of proper classification of many of the genotypes currently been cultivated in Mexico. The *A. caudatus* and *A. pumilus* genotypes provided by the Czech Republic also produced unexpected phenotypes, which suggested they were not properly classified.

Monitoring of pests, disease and weeds

The main pests and weeds, which were observed in the Czech Republic during field trials, are shown in Table 1.4.2. *Amaranthus sp.* is not a native European species and the cultivation is not widespread; this is the reason why the occurrence of pests and diseases in the Czech Republic is below the economic importance threshold. As a main pest, at both sites in the Czech Republic, the flea beetle (*Haltincinae*) was identified, which made small holes in the leaves in the stage from two to six real leaves. Damage was found sporadically, caused by other insects such as *Auchenorrhyncha*; it can be a vector for viruses which cause deformation of leaves. No pesticides were used during the field trials. In the Czech Republic, the problem with weeds occurred only before the leaves covered inter-row spaces. After shading of the soil surface by amaranth leaves the problem with weeds was reduced.

A survey of insect pests and beneficial insects that have a potential to be used for the biological control of amaranth pests, particularly Lepidoptera, was performed in Mexico during the 2007 and 2008 field trials. The type of insects recorded each year was similar, even though their population varied from year to year. The most abundant insect pests associated with the cultivation of grain amaranth in Central Mexico were: several species of Diptera; *Lebia spp.* (including *L. atriventris*); *Disonicha spp.*; *Diabrotica balteata* + *D. undecipunctata* + *D. virguifera*; *Colapsis spp.*; several species of Cicadellidae (nymphae + adults); *Microtalis spp.*; *Orius spp.*; *Lygus spp.*; several species of Pentatomidae (nymphae + adults); *Catorhinta guttula* (nymphae + adults); Earwigs (Dermaptera; Forficulidae; nymphae + adults); several species of Miridae (nymphae + adults); several species of Tingidae and Lepidoptera (mostly larvae). On the other hand, the most representative beneficial insects and associated fauna were the following: several species of Ephemeroptera ("mayflies"); many unidentified Hymenoptera, including many species of the Platygasteridae, Eurytomidae, Braconidae, Chalcididae, Sphecidae and Perilampidae; *Eupelmus sp.* (Hym; Eupelmidae); many species of Tachynidae (Diptera; Dip); numerous arachnids such as red mites, crab spider) and many unidentified arachnid species of all sizes); *Zelus spp.* (Reduviidae); several species of Reduviidae (Hemiptera; Hem); several species of Asilidae ("Robber flies"; Diptera) and Neuroptera (Chrysopidae); *Scymnus spp.* (Coleoptera; Coccinellidae); *Hipodamia convergens* (Coleoptera; Coccinellidae); *Cycloneda sanguinea* (Coleoptera; Coccinellidae) and *Collops spp.* (Coleoptera; Melyridae). Gram negative (i.e. *Pseudomonas argentinensis*, *Pantoea agglomerans* and *Microbacterium imperiale*) and Gram positive (i.e. Phytoplasmas) were isolated from diseased amaranth plants in the field and were associated with several disease symptoms such as chlorosis, dwarphism, leaf necrosis and others.

In Argentina, weed census was conducted at the end of each crop growing period; an estimated coverage of the soil by weeds was higher than 95%. All the weeds were identified, and the density and coverage area for each of them was estimated using the method of J. Braun Blanquet. The most important weeds were *Digitaria sanguinalis*, *Panicum capillare*, *Salsola kali* and *Chenopodium album*. Sampling of arthropod pests as well as beneficial insects was conducted weekly throughout the development of the crop in all treatments. In the state of seedling the phytophage species with the highest population density was *Acromyrmex striatus* (Roger) (Hymenoptera; Formicidae). During vegetative stages the most abundant species were *Epicauta adspersa* (Kluj) (Coleoptera; Meloidae), *Nezara viridula* (L.) and *Edessa meditabunda* (F.) (Hemiptera; Pentatomidae); in all stages a reproductive population of *Tetranychus urticae* (Koch) (Acari; Tetranychidae) was found.

In the trials performed in Spain there were no pests, diseases or weeds other than those present in the rest of summer field crops in the area. *Cryptocephalus sp.* attacks were observed in the first year and attacks of *Colaspidema atrum* in the second. In both cases they affected the plants in their early development stages, and they can cause significant defoliation. Both are controlled with a pyrethrin treatment. The disease did not have a particular impact during the development of the trials. Neither virus disease was a limiting factor for the crop

Estimation of the costs of amaranth production

In Argentina, considering standard farm equipment and cultivation practices (disk plow, spike and disk harrow, seeder) the indifference yield for any of the amaranth genotypes was about 800 kg/ha. Direct costs for the two years were around 150 US$ per hectare (indirect costs such as harvest, transport, taxes, etc. were also considered for gross margin calculation) and grain price was estimated in 1000 US$ per tonne. Gross margins ranged from 378 US$ per ha to 1174 US$ per ha at yields of 800 and 2000 kg/ha respectively. Even the lowest yielding genotypes (8 and 10) were slightly above indifference yield during the 2-year trial.

Production costs in irrigated amaranth in Spain are estimated at 994 €/ha. Estimating a production of 2 tonnes/ha its benefits can be obtained from 500 €/tonne of yielded grain. The current market prices for amaranth grain value is 800 to 1000 €/tonne, so the benefits can range from 1006 to 1506 €/ha. The gross profit is calculated on the basis of sales price of 800 €/tonne.

Besides these economic benefits, we should take into account the agronomic benefits that the crop can produce. These benefits are difficult to quantify. Thus, during the project development it was observed that some varieties have a very short cycle, which can be very well adapted to the typical crop rotation in the area, occupying the land during the summer months between two winter cereal crops. No other crop possible applications were taken into account, such as forage or industrial biomass production. These applications are, however, also interesting to consider in amaranth crops because they are interesting in the current agricultural situation.

The production costs in the Czech Republic were determined for low-input, standard, and intensive cultivation practices. In all cultivation practices costs were calculated included tillage, harvest, manpower, depreciation of machinery, seed prices, prices of fertilisers and plant protection (only in case of intensive and standard cultivation practices). In case of the Czech

Republic, three different purchase prices were used in calculations. From the results it is clear that the amaranth cultivation could be profitable in all cultivation practices. Only in the case of lower purchase price and in yield per ha about 1 tonne it is unprofitable. At the lowest purchase price, the yield should be higher than 1.5 tonne per ha to cover all the cultivation costs.

Table 1.4.1. Selected amaranth genotypes for field trials at all sites.

1 *A. cruentus* Mexicano (Origin: INTA Anguil, L.P.)
2 *A. cruentus* R127 (Origin: CRI, Czech Republic)
3 *Amaranthus* sp. K340 (Origin: CRI, Czech Republic)
4 *A. cruentus* Amont (Origin: CRI, Czech Republic)
5 *A. cruentus* CAC 48A (Origin: CRI, Czech Republic)
6 *A. cruentus* Don Guiem (Origin: INTA Anguil, L.P.)
7 *A. hypochondriacus* Revancha tipo Mercado (Origin: Mexico)
8 *A. hypochondriacus* Nutrisol Morfotipo Azteca (Origin: Mexico)
9 *A. cruentus* Tarasca (Origin: Mexico)
10 *A. hypochondriacus* Criollo Morelos (Origin: Mexico)
11 *A. hybridus* K 593 (Origin: CRI, Czech Republic)
12 *A. hypochondriacus* 280 FK-FH1 (Origin: CRI, Czech Republic)
13 *A. cruentus* cv. Don Leon
14 *A. cruentus* Candil (Origin: INTA Anguil, L.P.)
15 *A. hypochondriacus* Criollo San Antonio (Origin: Mexico)
16 *A. hypochondriacus* Criollo Rosita (Origin: Mexico)
17 *A. mantegazzianus* Don Juan (Origin: INTA Anguil, L.P.)
18 *A. hypochondriacus* Artasa 9122 (Origin: INTA Anguil, L.P.)

Table 1.4.2. Pests and weeds in field trials in the Czech Republic.

Pest	**Weed**
Haltincinae	*Echinochloa crus-gallii*
Agrotis segetum	*Amaranthus retroflexus*
Larvae Elateridae	*Portulaca oleracea*
Auchenorrhyncha	*Brassica sp.*
Pheasant	*Capsula bursa pastoris*
Hare	*Sonchus sp.*
	Chenopodium sp.
	Veronica sp.
	Solanum nigrum
	Plantago sp.

1.4.4 Impact of the project

Amaranth cultivation presents an attractive alternative for smallholder farmers in many parts of the world due to the relatively high price of amaranth. When we initiated the project, very little was known about the agronomic traits of the innumerable cultivars that exist in different parts of the world. Our field trials identified the genetic materials most adapted to Central and Southern Europe (the Czech Republic and Spain) as well as to Central and South America (Mexico and Argentina). We developed cultivation practices that are recommendable for high input technology production in the Czech Republic, Spain and Argentina (Repollo *et al.,* 2010;

Sánchez *et al.*, 2007; Taberner *et al.*, 2009). Another set of recommendations was produced for subsistence agriculture (Nicaragua) and smallholder agriculture (Mexico) where cultivation depends on mainly manual labour without technological inputs. Key irrigation is needed at the time of emergence. Then amaranth behaves as a typically local crop with low water requirements. Our results showed that for both contrasting production systems amaranth is an attractive and feasible alternative for food production and as a commodity for industrial transformations (Labouriau *et al.*, 2010a; 2010b; 2010c; 2010d).

1.5 Genetic and proteomic analysis of resistance and tolerance of amaranth towards important insect pests and diseases with the use of molecular markers

1.5.1 Objective

This research area included the genetic and proteomic analysis of resistance and tolerance of amaranth towards important insect pests and diseases with the use of molecular markers. The objective was further subdivided into three main tasks: **1)** identification and characterisation of genes leading to the synthesis and regulation of betacyanins in amaranth and their possible role in constitutive and induced insect resistance; **2)** identification and characterisation of genes involved in carbohydrate (CHO) metabolism, phloem loading, transport and signalling and their possible role in CHO re-allocation and ensuing tolerance to insect herbivory and **3)** identification and characterisation of genes and proteins involved in the defence mechanisms of amaranth to microbial infection and insect herbivory.

1.5.2 Methodology

A battery of genomic, proteomic and biochemical methodologies were utilised in this study (Navarro-Meléndez *et al.*, 2008; 2009; Ochoa-Sánchez *et al.*, 2009; Parra-Cota *et al.*, 2009; Vargas-Ortiz *et al.*, 2008; 2009; 2010). Genomic methods included: **1)** PCR amplification of candidate genes, utilising specially designed *primers* whose sequence was determined on the basis of conserved domains present in similar genes already reported for other plant species of the Cariophyllales (the order in which the Amaranthaceae plants are classified), as first choice, or in other orders or families; **2)** generation of *Suppressive Subtractive Hybridisation* (SSH) cDNA libraries, comprised of genes which are differentially expressed in a given condition (e.g. physiological, metabolic, developmental, environmental, etc.) but not in others, and tissue-specific (e.g. root, leaves or stems) combined with treatment-specific (e.g. application of abiotic and biotic stress) cDNA libraries and **3)** massive transcriptome sequencing. The above were utilised to isolate genes involved in pigment synthesis, in carbohydrate metabolism, transport and signalling and in responses to insect herbivory and bacterial infection. The proteomic approach involved the identification of proteins accumulating in response to insect herbivory and/or chemical inducers of an herbivory response, such as methyl jasmonate. These were isolated from proteomic maps resulting from the two-dimensional separation of proteins by isoelectric point (first dimension) and molecular weight (second dimension), respectively. Biochemical methods included the measurement of carbohydrate levels (mostly sucrose, glucose, fructose and starch) and sucrolytic activity (e.g. invertases and sucrose synthases) by means of highly specific enzyme-based assays. The latter results were mainly utilised to test the hypothesis that tolerance to insect herbivory damage in grain amaranth is related to the re-allocation of photosynthates from highly vulnerable (e.g. leaves) to less vulnerable (e.g. stems, roots) tissues, respectively.

1.5.3 End results

Identification and characterisation of genes involved in the synthesis of betacyanins in *Amaranthus spp.*

The PCR approach permitted the isolation of a partial cDNA coding for betanidin-O-5-glucosyl transferase. In addition, a partial cDNA coding for a small fragment of DOPA-4, 5-dioxygenase was obtained from the massive transcriptome sequencing of *A. hypochondriacus*. Interestingly,

this was the only experimental approach yielding a positive result for this particular gene, a condition that suggests that DOPA dioxygenase(s) in amaranth might greatly differ from those present in related species (e.g. sugar beet, spinach, *Chenopodium spp.*). The above two genes are considered to codify for key regulatory enzymes in the biosynthetic scheme of betacyanins, which is shown in Figure 1.5.1. A phylogenetic analysis of the above cDNA sequences grouped them with members found in closely related species (Figures 1.5.2 and 1.5.3).

Identification and characterisation of genes involved in carbohydrate metabolism, sink/source relationships, sugar transport and/or sugar signalling in *Amaranthus spp.* and also in CHO re-allocation to less vulnerable tissues in response to insect herbivory

The above experimental strategies permitted the isolation of partial cDNA sequences coding for one cell wall and vacuolar invertase, respectively, two cytoplasmic invertases, four sucrose synthases and one putative invertase inhibitor (Table 1.5.1). In addition, cDNAs coding for genes involved in starch (e.g. an ADP-glucose pyrophosphorylase subunit) cellulose biosynthesis, cell wall modification and CHO transport were similarly isolated (Table 1.5.2). Ongoing efforts to obtain the full cDNA sequences are now in progress. Short- and long-term changes in plant fitness and in sucrolytic activity, CHO and nitrogen levels in sink and source tissues of amaranth plants recovering from partial and temporal insect herbivory were recorded and are shown in Figures 1.5.4 to 1.5.9. The latter data revealed herbivory causes early changes in sucrolytic activity, intermediate to late changes CHO allocation to storage tissues and that loss of foliar tissue in an early stage of development has a deleterious effect on the fitness of the plant and suggest that yields will be positively affected if plants are protected from insect damage during cultivation.

Identification of genes and proteins involved in resistance responses to microbial infections and/or insect herbivory

Resistant (*A. cruentus* cv. *Tarasca*) and susceptible (*A. hypochondriacus* cv. *Revancha*) genotypes towards infection with *Pseudomonas argentinensis*, a bacterial pathogen isolated from diseased plants in the field, were identified (Casarrubias-Castillo, 2009). This difference was exploited to prepare SSH libraries, from which several defence genes were identified (Table 1.5.3). The results suggest that resistance to *P. argentinensis* in *A. cruentus* is dependent on both jasmonic acid- and salicylic acid-regulated signalling pathways. Also, foliar protein profiles were compared after exogenous methyl jasmonate (MeJA) treatments or herbivory assays with *Spodoptera exigua* larvae in plants of *A. hypochondriacus*. Both treatments showed the accumulation of differential proteins of low molecular weight, in the acidic and basic range: approximately 513 protein spots by hervibory elicitation (Figure 1.5.10) and 343 spots by MeJA treatment. A subtractive library based on herbivory was used in an effort to match genes to unidentified protein sequences (Table 1.5.3). In some cases the sequences of induced genes were consistent with those of differential proteins. In general, proteins and genes that were up-regulated were involved in primary metabolism, transcriptional and translational regulation and defence. Examples of the latter were the chloroplastic ATP synthase γ-subunit and harpin, involved in the plant perception of herbivory (as inceptin peptides), and in the elicitation of the hypersensitive response (HR), respectively (Délano-Frier, 2008; 2009a; 2009b).

1.5.4 Impact of the project

A sizeable proportion of the results presented here are described for the first time. This is not surprising, considering the scarce molecular and biochemical information that is available regarding key aspects of amaranth physiology, including CHO metabolism and re-allocation and defence responses, including the possible role of pigment accumulation, against biotic aggressors such as pathogenic bacteria and chewing insects. The data presented establish a solid point of reference for future investigations, many of which are still being pursued by the research groups involved in the Amaranth Future Food project. The knowledge will surely shed new light on the mechanisms of resistance and/or tolerance against biotic stress in amaranth and will undoubtedly offer tools for the continued improvement of amaranth as a commercial crop.

Table 1.5.1. Sucrolytic cDNA sequences isolated from *A. cruentus* and *A. hypochondriacus*.

Enzyme cloned	Function	Number of sequences isolated	Sequence size or size range (base pairs)	Length of missing fragments (base pairs)
Cell wall invertase	Control of sucrose distribution between sink and source tissues. Inducible by pathogens and herbivores	1	854	To 3' end: 700 To 5' end: 600
Vacuolar invertase	Osmoregulation. Control of cell expansion and of sugar composition in storage tissues	1	720	To 3' end: 900 To 5' end: 400
Cytosolic invertase	Cell maintenance. Growth and development control in Arabidopsis and *Lotus japonicus*	2	241-650	To 3' end: 100 To 5' end: 900
Sucrose synthase	Control of sucrose distribution between sink and source tissues. Inducible by low temperatures and anaerobiosis. Synthesis of UDP-glucose required for cellulose and/or starch biosynthesis	4	200-450	To 3' end: 1500 To 5' end: 600

Table 1.5.2. Amaranth cDNA sequences involved in cell wall synthesis and re-arrangement, sugar metabolism, reallocation and signalling.

Gene domain	Gene description	Gene function	Known sequences
Left-handed parallel beta-Helix (LbetaH or LbH) domain	ADP-glucose pyrophosphorylase	Starch synthesis	*Spinacia oleracea*
	GDP-L-galactose phosphorylase	Smirnoff-Wheeler Pathway to **Ascorbic Acid** in plants; seedling viability	*Malpighia glabra*
	Arabinogalactan protein, **Structural cell-wall proteins**	Histidine-Rich Extensin from **Zea mays** is an **Arabinogalactan protein**	*Zea mays*
rft1, putative. Flippases Distinct Flippases Translocate Glycerophospho- lipids and Oligosaccharide Diphosphate Dolichols across the Endoplasmic Reticulum	Oligosaccharide translocation protein; Nuclear division RFT1-like protein	The glycolipid $Glc_3Man_9GlcNAc_2$-PP-dolichol is the oligosaccharide donor for protein N-glycosylation reactions in the ER lumen	*Ricinus communis, Sisymbrium irio* (Cruciferae)
UAA transporter family. This family includes transporters with a specificity for UDP-N-acetyl glucosamine	ATUTR5/UTR5 (UDP-GALACTOSE TRANSPORTER 5); galactose transmembrane transporter	The folding of glycolproteins in the endoplasmic reticulum (ER) depends on a quality control mechanism mediated by the calnexin/calreticulin cycle. During this process, continuous glucose trimming and UDP-glucose-dependent re-glucosylation of unfolded glycoproteins take place	*Arabidopsis thaliana*
Cellulose synthase: function designated as the "cellulose synthase-like" genes (CslA, CslB, CslC, CslD, CslE and CslG). It is possible that these cellulose synthase like (Csl) proteins do not contribute to cellulose synthesis, but rather to the synthesis of other wall polymers	More correctly designated as 'cellulose synthase catalytic subunits', plant cellulose synthase (CesA) proteins are integral membrane proteins, approximately 1,000 amino acids in length. There are a number of highly conserved residues, including several motifs shown to be necessary for processive glycosyltransferase activity	**Cellulose synthase**, putative; **Cellulose synthase-like** protein CslG	*Ricinus communis; Nicotiana tabacum*
Alpha-L-arabinofuranosidase C-terminus //Hvad med dette tegn?This family represents the C-terminus (approximately 200 residues) of bacterial and eukaryotic alpha-L-arabinofuranosidase (EC:3.2.1.55)	This catalyses the hydrolysis of nonreducing terminal alpha-L-arabinofuranosidic linkages in L-arabinose-containing polysaccharides. **Cell wall modifications in arabidopsis plants with altered {alpha}-l-arabino furanosidase activity.** Alpha-L-arabinofuranosidases (a-Afs) are plant enzymes capable of releasing terminal arabinofuranosyl residues from cell wall matrix polymers	Putative alpha-L-arabino furanosidase. Galactose-binding-like; alpha-L-arabinofuranosidase, C-terminal	*Arabidopsis thaliana. Me dicago truncatula*
Putative glycosyltransferase	Galactosyl transferase GMA12/MNN10 family. Some members of this family are included in glycosyltransferase **family 34**		*Lotus japonicus*

Gene domain	Gene description	Gene function	Known sequences
PMEI. Plant invertase /pectin methylesterase inhibitor	It has been implicated in the regulation of fruit development, carbohydrate metabolism and cell wall extension (see Henvisning?). It may also be involved in inhibiting **microbial pathogen PMEs**		
GT1_Glycogen_synthase_DULL1-like	Granule-bound glycogen (**starch) synthase**	Five SS isoforms, SSI, II, III, IV and **Granule Bound SSI**, have been identified, each with a unique catalytic role in starch synthesis	*Astragalus membranaceus* (Chinese medicinal herb)
GDP-mannose pyrophosphorylase	GDP-mannose pyrophosphorylase (mannose-1-phosphate guanyltransferase), synthesises GDP-mannose from GTP and mannose-1-phosphate in **cell wall biosynthesis**; required for normal **cell wall structure**		*Solanum tuberosum*
Xyloglucan endotransglycosylase/hydrolase precursor XTH-3	Glyco_hydrolase_16. **Family 1**6 includes lichenase, xyloglucan endotransglycosylase (XET), beta-agarase, kappa-carrageenase, endo-beta-1,3-glucanase, endo-beta-1,3-1,4-glucanase, and endo-beta-galactosidase	**Xyloglucan endotrans glycosylases (XETs)** cleave and religate xy loglucan polymers in plant cell walls via a transglycosylation me chanism. **Thus, XET is a key enzyme in all plant processes that require cell wall remodelling**	*Populus tremula* x *Populus tremuloides*
Xyloglucan endotransglycosylase 7	Family 16		*Arabidopsis thaliana* *Pisum sativum*
The X8 domain	The domain is found in an olive pollen allergen as well as at the **C-terminus of family 17 glycosyl hydrolases.** Isozymes of glycosyl hydrolase Family 17 hydrolyse 1,3-beta-glucan polysaccharides found in the cell wall matrix of plants and fungi	Carbohydrate binding. Predicted **GPI-anchored** protein	

Table 1.5.3. Genes isolated from SSH libraries comparing resistant versus susceptible genotypes in terms of bacterial infection, herbivory damage and stress responses.

Gene	Description	Example/Type	Species
Function: Unknown: Domain of Unknown Function (DUF)			
DUF6	This family includes many hypothetical membrane proteins of unknown function		
DUF246	Possible involvement in auxin-independent growth regulation, on the basis of publications which have been retracted	Chloroplast phosphate translocator protein	Spinach
DUF296	Contain AT-hook motifs which strongly suggest a DNA-binding function. The MADS box genes encode a eukaryotic family of transcriptional regulators involved in diverse and important biological functions	**SAP1** protein	*Antirrhinum majus* (flowering)
	Putative senescence-associated protein	**SAP1**	*Cupressus sempervirens*, *Sarracenia purpurea*, *Picea abies*
	Stress-associated protein 6 (SAP6) gene: environmental stress-responsive SAP gene family encoding A20/AN1 zinc finger proteins in tomato	**SAP 6**	*Solanum pennellii, Gossypium hirsutum*
DUF538	Hypothetical protein.		*Vitis vinifera*
DUF640	Light sensitive hypocotyls 10	Functionally dependent on phytochrome to me-diate light regulation of seedling development	*Arabidopsis thaliana*
DUF760	Cassava lambda zap cDNA		*Manihot esculenta*
DUF829	This family consists of several uncharacterised eukaryotic proteins		
cDNA clone C05803A07	Leaves from plants exposed to iron deficiency for 1 year		*Citrus clementina*
Function: Water, osmotic (salt), UV-light and other abiotic stresses			
DUF221	This family consists of hypothetical trans-membrane proteins none of which have any function; the aligned region is at 538 residues at maximum length	**ERD4** protein; early-responsive to dehydration stress)	
ERD15		**ERD15 (early responsive to dehydration 15).**	*Capsicum annuum*
Aldehyde dehydrogena-se family	Betaine aldehyde dehydrogenase	ahybadh4	***Amaranthus hypochondriacus***

Gene	Description	Example/Type	Species
Amino acid permease	Proline/glycine betaine transporter	In mammalian cells and microorganisms such as *Escherichia coli*, choline dehydrogenase (CDH) and betaine aldehyde dehydrogenase (BADH) catalyse the oxidative reaction via two steps: choline → betaine aldehyde → glycine betaine. In higher plants, the pathway is the same, with choline monooxygenase (CMO) catalysing the first step instead of CDH. CMO activity is very restricted to plant species and is only found in the Chenopodiaceae and the Amaranthaceae	*Atriplex hortensis*
Highly conserved protein containing a thioredoxin domain, [posttranslational modification, protein turnover, chaperones]	Cold-induced **thioredoxin** domain-containing protein		***Ammopiptanthus mongolicus:*** a ligneous plant, it can survive at -30°C or even lower temperatures in winter. Besides its remarkable freezing tolerance, *A. mongolicus* has drought, salt, and alkali resistant capabilities
HSP21 (HEAT SHOCK PROTEIN 21) (HSP21)	**Alpha-crystalline domain ACD**		***Ammopiptanthus mongolicus* (Fabaceae); *Arabidopsis thaliana***
Protein disaggregation chaperone; provisional	Heat shock protein		*Solanum lycopersicum*
Hsp70 chaperones	HOT stress. Hsp70 activity is ATP dependent. Hsp70 proteins are made up of two regions: the amino terminus is the ATPase domain and the carboxyl terminus is the substrate binding region	Hsp70	*Musa acuminata* subsp. Burmannicoides (leaves). The wild diploid genotype *Musa acuminata* subsp. *burmannicoides*, var. Calcutta 4 shows considerable resistance to fungal pathogens and represents a potential source of resistance related genes
Cytosolic heat shock 70 protein (HSC70-1)			***Spinacia oleracea***
DnaJ domain or J-domain. DnaJ/Hsp40 (heat shock protein 40)	They act primarily by stimulating the ATPase activity of **Hsp70s**, an important chaperonine family. Hsp40 proteins are characterised by the presence of a J domain, which mediates the interaction with Hsp70	DnaJ-like protein isoform	*Solanum phureja*
Chaperonin-like superfamily	Chaperonins are involved in productive folding of proteins	Clone 12972 mRNA, complete sequence	*Arabidopsis thaliana*

Gene	Description	Example/Type	Species
Histidine kinase-like ATPases	This family includes several ATP-binding proteins for example: histidine kinase, DNA gyrase B, topo isomerases, **heat shock protein HSP90**, phytochrome-like ATPases and DNA mismatch repair proteins	An expressed sequence tag database for abiotic stressed berries of *Vitis vinifera* var. Chardonnay *Vitis vinifera* cDNA clone VVD017A03 5, mRNA sequence	*Vitis vinifera*
Sulfate transporter; salt stress	Expression library, **5 days 0.5 M NaCl treatment; Crassulacean acid metabolism, phase IV** (5:30 PM)	The STAS (after Sulphate Transporter and AntiSigma factor antagonist) domain is found in the C terminal region of sulphate transporters and bacterial antisigma factor antagonists	*Populus alba x Populus tremula; Mesembryanthemum crystallinum* (ice plant); Order Caryophyllales
Salt stress	Expression library, **48 hours NaCl treatment**		*M. crystallinum*
Salt stress	Expression library, **0 hours NaCl treatment**		*M. crystallinum*
Rieske domain	Expression library, **0 hours NaCl treatment**	[2Fe-2S] cluster binding domain commonly found in Rieske non-heme iron oxygenase (RO) systems such as naphthalene and biphenyl **dioxygenases**, as well as in plant cyano bacterial **chloroplast b6f** and mitochondrial cytochrome bc(1) complexes	*M. crystallinum*
Methyltransferase (salt stress)	Expression library, **5 days 0.5 M NaCl treatment; Crassulacean acid metabolism, phase I (2 AM)**	Bifunctional N5-glutamine S-adenosyl-L-methio nine-dependent methyl transferase/tRNA	*M. crystallinum*
Cyclophilins are a diverse family in terms of function and have been implicated in protein folding processes which depend on catalytic /chaperone-like activities	Expression library, **0 hours NaCl treatment**	Cyclophilin: cyclophilin-type peptidylprolyl cis-trans isomerases	*M. crystallinum*
Thioredoxin-like protein	Expression library, **5 days 0.5 M NaCl treatment; Crassulacean acid metabolism, phase IV (5:30 PM)**		*M. crystallinum*
Zeta-carotene desaturase	Expression library, **0 hours NaCl treatment;** M. crystallinum	The **carotenoids** can quench harmful ROS generated from **water** radiolysis	*Linum usitatissimum*
Salt stress	Water use efficient species. Commonly called Old Man Saltbush, is a member of the saltbush family, Chenopodiaceae. Usually found on saline, clay soils in low-lying areas	Salt-induced hydrophilic protein	*Atriplex nummularia*; Order Cariophyllales, Amaranthaceae
Salt stress	**Salt-induced hydrophilic protein**	Salt-induced hydrophilic protein (AnSIHP1 mRNA)	*Atriplex nummularia*
The Major Facilitator Superfamily (MFS)	Subtractive cDNA library specific for UV-B irradiated lettuce leaves		*Lactuca sativa*

Gene	Description	Example/Type	Species
UV-B irradiated lettuce leaves	**Subtractive cDNA library specific for UV-B irradiated lettuce leaves cDNA clone PJS-07-B01**		***Lactuca sativa***
MIP. Major intrinsic protein (MIP) superfamily	Members of the MIP superfamily function as membrane channels that selectively transport water, small neutral molecules and ions out of and between cells	The superfamily can be subdivided into two major groups: one is the water selective channels called **aquaporins (AQPs)**	*M. crystallinum* major intrinsic protein homolog (**mipD**) mRNA
Cation efflux family	Members of this family are integral membrane proteins that are found to increase tolerance to divalent metal ions such as cadmium, zinc and cobalt. These proteins are thought to be efflux pumps that remove these ions from cells	Putative Zn transporter	*Hordeum vulgare*
	Nitrate chlorate transporter 1.1		*Fagus sylvatica* (Haya)
	Constitutively_frost_tolerant Brassica napus cDNA clone Bn01		*Brassica napus*
S-adenosyl-L-methio nine synthase 2	Ethylene? Lignin? Biosynthesis		
Super-family Isocitrate/ isopropyl-malate dehydrogenase. Isocitrate de hydrogenase, putative	cDNA cold-acclimated Bluecrop library		*Vaccinium corymbosum* (highbush blueberry) *Ricinus communis*
Metallothionein	**Metallothionein** (MT) is a family of cysteine-rich, low molecular weight (MW ranging from 3500 to 14000 Da) proteins. MTs have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which represents nearly the 30% of its amino acidic residues		*Porteresia coarctata* (halophytic wild rice; mangrove communities) ***Amaranthus cruentus***
Metallothionein	These proteins are cysteine rich proteins that bind to heavy metals, Cu, Zn, Cd, Hg, Ag. Members of this family appear to be closest to Class II metallothioneins		***Amaranthus cruentus***
Transmembrane transport protein-like protein			*Arabidopsis thaliana*
UVB-resistance protein-like	Alpha-tubulin suppressor and related RCC1 domain-containing proteins [Cell division and chromosome partitioning / Cytoskeleton]		
Function: Oxidative stress, Redox state			
	Multicopper oxidase, putative	Pollen-specific protein-like **predicted GPI-anchored protein**	*Ricinus communis*
	Hydrogen peroxide-induced 1	Solanum habrochaites cDNA clone LH	*Nicotiana tabacum*
Dim1 family Thioredoxin-like, with user query added Superfamily // Protein Disulfide Oxidoreductases and other proteins with a Thioredoxin fold	They function as protein disulfide oxidoreductases (PDOs), altering the redox state of target proteins via the **reversible oxidation of their active site dithiol**	Thioredoxin-like protein 4A, putative, expressed	*Oryza sativa* (japonica cultivar-group)

Gene	Description	Example/Type	Species
Glutathione S-transferase (GST) family	**Sugar beet peroxide germination cDNA library (subtracted)**	Diverse group of cytosolic dimeric proteins involved in cellular detoxification by catalysing the conjugation of glutathione (GSH) with a wide range of endogenous and xenobiotic alkylating agents, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress	***Beta vulgaris***
Germin-like protein (GLP3b)	Cupin domain. This family represents the conserved barrel domain of the 'cupin' superfamily	GER3 (GERMIN 3); oxalate oxidase	*Arabidopsis thaliana*
PLANTACYANIN; Cu_bind-like, Plastocyanin-like domain	REDOX PROPERTIES OF THE BASIC BLUE PROTEIN (PLANTACYANIN). The available structural data suggest that stellacyanins (and possibly other phytocyanins) might not be diffusible electron-transfer proteins participating in long-range electron-transfer processes. Conceivably, they are involved in redox reactions occurring during primary defence responses in plants and/or in lignin formation		*Spinacia oleraceae*
sks5 (SKU5 Similar 5); copper ion binding/ oxidoreductase (sks5) mRNA, L-ascorbate oxidase precursor [multicopper oxidase, putative]	Pectin esterase, putative		*Arabidopsis thaliana, Zea mays, Ricinus communis*
Cationic peroxidase (POD6) mRNA, Cationic peroxidase 2	Along with animal peroxidases, these enzymes belong to a group of heme-dependent peroxidases containing a heme prosthetic group (ferriproto porphyrin IX), which catalyses a multistep oxidative reaction involving hydrogen peroxide as the electron acceptor. The plant peroxidase superfamily is comprised of three structurally and functionally divergent groups	Ten *POD* genes, including three ascorbate peroxidases (class I *POD*s) and seven secretory peroxidases (class III *POD*s), were cloned from *Tamarix hispida*	***Tamarix hispida*** (a woody halophyte)(Cariophyllales); Glycine max

Gene	Description	Example/Type	Species
GLYOXALASE I HOMOLOG; Dioxygenase superfamily. Dioxygenases are nonheme iron-containing enzymes important in the biosynthesis of plant signalling compounds such as abscisic acid, gibberellins and ethylene and also of secondary metabolites, notably flavonoids and alkaloids. Plant dioxygenases fall into two classes: lipoxygenases and 2-oxoacid-dependent dioxygenases. The latter catalyse hydroxyllation, epoxidation and desaturation reactions; some enzymes catalyse more than one type of reaction in successive steps in a biosynthetic pathway. This review highlights recent discoveries on both enzyme groups, particularly in relation to gibberellin biosynthesis, in vivo activity of 1-aminocyclopropane-1-carboxylate oxidase and molecular structure/function relationships	Glyoxalase I (EC 4.4.1.5) activity has long been associated with rapid cell proliferation, but experimental evidence is forthcoming, linking its role to **stress tolerance** as well	Methylglyoxal is metabolised to lactic acid by two different routes. One route is a glyoxalase system in which glyoxalase I and glyoxalase II are involved. In this route, methylglyoxal is condensed with glutathione to give *S*-D-lactoyl glutathione by the action of glyoxalase I, and the glutathione thiolester is then hydrolysed to lactic acid and glutathione by glyoxa lase II	*Arabidopsis thaliana, Zea mays, Oryza sativa*
Fe-superoxide dismutase 1 precursor (sodB) mRNA	Iron/manganese superoxide dismutases, C-terminal domain	Superoxide dismutases (SODs) catalyse the conversion of super oxide radicals to hydrogen peroxide and molecular oxygen. Three evolutionarily distinct families of SODs are known, of which the Mn/Fe-binding family is one	*Lotus japonicus*
Function: Defence and biotic stress			
Tomato mixed elicitor	Plants exposed to 2,6 dichloroisonicotinic acid, BTH, jasmonic acid, ethylene, fenthion, EIX, okadaic acid or systemin prior to tissue harvest	**BTI**	*Lycopersicon esculentum*
Elicitor inducible beta-1,3-glucanase	Glycosyl hydrolase family 17 protein	X8 domain: The X8 domain contains 6 conserved cysteine residues that presumably form three disulphide bridges	*Nicotiana tabacum, Arabidopsis thaliana*
ClpC protease (clpC)	Clp protease ATP-binding subunit clpA homolog CD4B, chloroplastic		*S. oleraceae*
ClpC protease	ATP-dependent Clp protease proteolytic subunit		*Capsicum annuum; Malus x domestica*
MATE (multidrug and toxin extrusion) trans porter family	EDS5 is homologous with members of the MATE (multidrug and toxin extrusion) transporter family *EDS5* expression is very low in unstressed plants and strongly induced by **pathogens** and **UV-C** light		Unknown protein [*Arabi dopsis thaliana*]
Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family	Proteins in this family are known to play important roles in defending plants from insects and pathogens, lipid transport between intracellular membranes and nutrient storage	These proteins contain a common pattern of eight cysteines that form four disulfide bridges (¿knottins?)	

Gene	Description	Example/Type	Species
The Amaranth Alpha-Amylase Inhibitor gi|15826209|pdb|1HTX|A Chain A, Solution Structure of the Main Alpha-Amylase Inhibitor From Amaranth Seeds			
Nonspecific lipid-transfer protein precursor (LTP)	Plant nsLTPs are small, soluble proteins that facilitate the transfer of fatty acids, phospholipids, glycolipids and steroids between membranes. In addition to lipid transport and assembly, **nsLTPs also play a key role in the defence of plants against pathogens**		***Beta vulgaris* subsp. vulgaris**
Lethal leaf spot 1-like protein	The maize lethal leaf spot 1 (*lls1*) mutant exhibits enhanced resistance to fungal pathogens	*lls1* lesions express **pathogenesis-related proteins at high levels**, so lesion sterility from activation of defence systems and necrosis	*Lycopersicon esculentum*
Trypsin inhibitor, Soybean trypsin inhibitor (Kunitz) family	Inhibit proteases by binding with high affinity to their active sites	STI	*Herrania mariae*, ***Populus tremula***
Potato inhibitor I family			
Thiazole biosynthetic enzyme			**Cq seed *Chenopodium quinoa***
Glutamine amidotransferases class-II (GATase)	**Gm_ck25424 : induced by salicylic acid**		*Glycine max*
	Gm_ck8357: induced by salicylic acid		*Glycine max*
NDR1/HIN1-like protein 1	**This family contains a number of plant harpin-induced 1 (Hin1) proteins, which are involved in the plant hypersensitive response (HR)**	**Harpin-induced protein 1 (Hin1)**	*Arabidopsis thaliana*
Catalase	Protection of cells from the toxic effects of peroxides	**CAT1**	*Suaeda maritima* subsp. salsa. Halophytes, alkaline soils, Caryophyllales, Amaranthacea
Forisomes	**Forisome**s are Ca^{2+}-dependent contractile protein bodies that form reversible plugs in sieve tubes of faboid legumes	**Forisomes** represent a defence mechanism by reversibly plugging the sieve tubes in response to injury	*Canavalia gladiata, Ricinus communis, Populus trichocarpa*
Chitinase-like protein (CTL2) gene, class II chitinase	Family 19 chitinases are found primarily in plants (classes I, III, and IV). Class II chitinases lack both the chitin-binding domain and the hinge region		*Gossypium hirsutum, Pyrus pyrifolia* (Asian pear)

Gene	Description	Example/Type	Species
Function: Jasmonic acid, (oxi) Lipid-based signalling			
Serine incorporator (Serinc)	This is a family of eukaryotic membrane proteins which incorporate serine into membranes and facilitate the synthesis of the serine-derived lipids **phosphatidylserine** and **sphingolipid**	Members of this family contain 11 transmembrane domains and form intracellular complexes with key enzymes involved in **serine and sphingolipid** biosynthesis	*Ricinus communis*
GH3 auxin-responsive promoter. GH3 family protein	Jasmonic acid-amino acid-conjugating enzyme		*Populus trichocarpa, Nicotiana attenuata*
	Jasmonate-induced protein homolog; 3 independent sequences	Similar to barley jasmonate-induced protein	Barley
	Long-chain acyl-CoA synthetase	*Ricinus communis* ACS4 mRNA, complete cds	*Arabidopsis thaliana*
	Phospholipase C 3 precursor, putative		*Ricinus communis*
PLDP1 (PHOSPHOLIPASE D ZETA1)	Phospholipase D-like protein		*Arabidopsis thaliana*
Calmodulin-binding region; Fatty oxidation complex, alpha subunit FadJ. NADPH:protochlorophyllide oxidoreductase	**Calmodulin-binding protein**	Multifunctional anaerobic fatty acid oxidation complex FadIJ; has 3-hydroxyacyl-CoA dehydrogenase and medium-long-chain-length enoyl-CoA hydratase active ties; probable beta-hy droxybutyryl-CoA epimerase and dodecenoyl-CoA-delta-isomerase	*Medicago truncatula Nicotiana tabacum*
POR2 mRNA for NADPH-proto chlorophyllide oxidoreductase 2	Chlorophyll synthesis		***Amaranthus tricolor***
Lipolytic enzyme, G-D-S-L	**SGNH_hydrolase, or GDSL_hydrolase**, is a diverse family of lipases and esterases	Some of the GDSL enzymes have thioesterase, protease, arylesterase and lysophospholipase activity	*Medicago truncatula*
Lipoxygenase			Sugar beet field-harvested seed stalks ***Beta vulgaris*** cDNA
Agglutinin (Lectin)	Pore-forming toxin-like protein **Hfr-2**		***Amaranthus caudatus*** *Triticum aestivum*
SEC14. - Sec14p-like lipid-binding domain	Putative phosphatidyl-inositol-transfer protein		
Jasmonate-induced protein. Dominio Superfamilia jacalinas	**Jacalin-related lectin. The function of Horcolin is discussed in the context of its particular expression in coleoptiles and is then compared to other lectins, which apparently share a related response to biotic or abiotic stress factors**		***Prosthecochloris aestuarii****, Gossypium hirsutum, Triticum aestivum, Hordeum vulgare*
Mannose-specific recombinant lectin	jasmonate-induced protein Jacalin, Jacalin-like lectin domain OR En/Spm-like transposon		***Zea mays, Beta vulgaris***

Gene	Description	Example/Type	Species
Barley jasmonate-induced protein	Cysteine synthase, autoinhibited H+ ATPase		*Glycine max, Populus trichocarpa*
Glycine max clone gmw1-103e11, jasmonate-induced protein	**Jacalin, Jacalin-like lectin domain**		*Glycine max, Triticum aestivum*
Glycine max clone gmw1-103e11	**Jacalin, Jacalin-like lectin domain**		*Triticum aestivum,*
Populus trichocarpa clone Pop1-053A03	**Jasmonate-induced protein jacalin-lectin-like domain**		***Triticum aestivum***
Glycine max clone gmw1-103e11	**Jasmonate-induced protein jacalin lectin-like domain**		*Triticum aestivum*
Vegetative storage protein PNI288	Vegetative storage proteins (VSPs) are thought to play important roles in within-plant N cycling	Known poplar defence-related genes, including *win6.2C* and *win8* endochitinases, *PtdPPO1*, vegetative storage proteins (VSPs) *win4.5* and ***pni288*** and trypsin inhibitors (TIs)	*Populus balsamifera subsp. trichocarpa x Populus deltoides*
Cytochrome P450 fatty acid omega-hydroxylase	Omega-1-hydroxylase/ oxygen binding		*Arabidopsis thaliana Petunia x hybrida*
Function: Phytohormone signalling			
Auxin	Auxin-repressed protein-like protein ARP1	Dormancy/auxin associated protein. This family contains several plant dormancy-associated and auxin-repressed proteins the function of which is poorly understood	*Manihot esculenta*
Auxin	Auxin-repressed protein		*Arachis hypogaea*
Auxin	Auxin-binding protein ABP20 precursor	Cupin domain. This family represents the conserved barrel domain of the 'cupin' superfamily	*Prunus persica*
Auxin	Membrane transport protein. - This family includes **auxin efflux carrier proteins** and other transporter proteins from all domains of life	Putative sodium-dependent bile acid symporter	*Arabidopsis thaliana*
Auxin	**Auxin-induced protein 5NG4; nodulin MtN21 family protein**	Auxin-mediated signalling pathway; transport	*Ricinus communis Arabidopsis thaliana*
Function: Cell wall synthesis, re-arrangement. Sugar metabolism, reallocation, signalling			
Left-handed parallel beta-Helix (LbetaH or LbH) domain	ADP-glucose pyrophosphorylase	Starch synthesis	*Spinacia oleracea*
	GDP-L-galactose phosphorylase	Smirnoff-Wheeler pathway to **Ascorbic Acid** in plants; seedling viability	*Malpighia glabra*
	Arabinogalactan protein, **Structural cell-wall proteins**	Histidine-rich extensin from **Zea mays** is an **Arabinogalactan protein**	*Zea mays*

Gene	Description	Example/Type	Species
rft1, putative. Flippases Distinct Flippases Translocate Glycerophospholipids and Oligosaccharide Diphosphate Dolichols across the Endoplasmic Reticulum	Oligosaccharide translocation protein; nuclear division RFT1-like protein	The glycolipid $Glc_3Man_9GlcNAc_2$-PP-dolichol is the oligosaccharide donor for protein N-glycosylation reactions in the ER lumen	*Ricinus communis, Sisymbrium irio* (Cruciferae)
UAA transporter family. This family includes transporters with a specificity for UDP-N-acetyl glucosamine	ATUTR5/UTR5 (UDP-GALACTOSE TRANSPORTER 5); galactose transmembrane transporter	The folding of glycoproteins in the endoplasmic reticulum (ER) depends on a quality control mechanism mediated by the calnexin/calreticulin cycle. During this process, continuous glucose trimming and UDP-glucose-dependent re-glucosylation of unfolded glycoproteins takes place	*Arabidopsis thaliana*
Cellulose synthase: function designated as the "cellulose synthase-like" genes (CslA, CslB, CslC, CslD, CslE, and CslG). It is possible that these cellulose synthase- like (Csl) proteins do not contribute to cellulose synthesis, but rather to the synthesis of other wall polymers	More correctly designated as 'cellulose synthase catalytic subunits', plant cellulose synthase (CesA) proteins are integral membrane proteins, approximately 1,000 amino acids in length. There are a number of highly conserved residues, including several motifs shown to be necessary for processive glycosyltransferase activity	**Cellulose synthase**, putative; **Cellulose synthase-like** protein CslG	*Ricinus communis; Nicotiana tabacum*
Alpha-L-arabinofuranosidase C-terminus //This family represents the C-terminus (approximately 200 residues) of bacterial and eukaryotic alpha-L-arabinofuranosidase (EC:3.2.1.55)	This catalyses the hydrolysis of nonreducing terminal alpha-L-arabinofuranosidic linkages in L-arabinose-containing polysaccharides. **Cell wall modifications in arabidopsis plants with altered {alpha}-l-arabino furanosidase activity.** Alpha-L-arabinofuranosidases (a-Afs) are plant enzymes capable of releasing terminal arabinofuranosyl residues from cell wall matrix polymers	Putative alpha-L-arabino furanosidase. Galactose-binding-like; alpha-L-ara binofuranosidase, C-terminal	*Arabidopsis thaliana. Medicago truncatula*
Putative glycosyltransferase	Galactosyl transferase GMA12/MNN10 family. Some members of this family are included in glycosyltransferase **family 34**		*Lotus japonicus*
PMEI. Plant invertase /pectin methylesterase inhibitor	It has been implicated in the regulation of fruit development, carbohydrate metabolism and cell wall extension. It may also be involved in inhibiting **microbial pathogen PMEs**		
GT1_Glycogen_synthase_DULL1-like	Granule-bound glycogen (**starch) synthase**	Five SS isoforms, SSI, II, III, IV and **Granule Bound SSI**, have been identified, each with a unique catalytic role in starch synthesis	*Astragalus membranaceus* (Chinese medicinal herb)

Gene	Description	Example/Type	Species
GDP-mannose pyrophosphorylase	GDP-mannose pyrophosphorylase (mannose-1-phosphate guanyltransferase), synthesises GDP-mannose from GTP and mannose-1-phosphate in **cell wall biosynthesis**; required for normal **cell wall structure**		*Solanum tuberosum*
Xyloglucan endotransglycosylase/hydrolase precursor XTH-3	Glycohydrolase_16. **Family 1**6 includes lichenase, xyloglucan endotransglycosylase (XET), beta-agarase, kappa-carrageenase, endo-beta-1,3-glucanase, endo-beta-1,3-1,4-glucanase, and endo-beta-galactosidase	**Xyloglucan endotrans glycosylases (XETs)** cleave and religate xyloglucan polymers in plant cell walls via a transglycosylation mechanism. **Thus, XET is a key enzyme in all plant processes that require cell wall remodelling**	*Populus tremula x Populus tremuloides*
Xyloglucan endotransglycosylase 7	Family 16		*Arabidopsis thaliana* *Pisum sativum*
The X8 domain	The domain is found in an olive pollen allergen as well as at the **C-terminus of family 17 glycosyl hydrolases.** Isozymes of glycosyl hydrolase family 17 hydrolyse 1,3-beta-glucan polysaccharides found in the cell wall matrix of plants and fungi	Carbohydrate binding. Predicted **GPI-anchored** protein	
Function: Protein hydrolysis, protein turnover, proteasome, protein traffic, senescence			
Eukaryotic aspartyl protease aspartyl (acid) proteases include pepsins, cathepsins and renins	Aspartic protease precursor		*Lycopersicon esculentum*
N-terminal nucleophile (NTN-) hydrolase superfamily, which contains a four-layered alpha, be- ta, beta, alpha core structure	Proteasome subunit alpha type, putative; 20S proteasome subunit **PAF1**		*Ricinus communis*
Vesicle transport v-SNA RE protein	V-SNARE proteins are required for protein traffic between eukaryotic organelles. The v-SNAREs on transport vesicles interact with t-SNAREs on target membranes in order to facilitate this	Golgi snare 11 protein, putative	*Ricinus communis, A. thaliana*
Ubiquitin-Associated domain. The UBA domain is a commonly occurring sequence motif in some members of the ubiquitination pathway, UV excision repair proteins and certain protein kinases	Plant ubiquilin, putative	**Sugar beet** 10-week GH root cDNA cDNA 5', mRNA sequence	*Beta vulgaris*
Proteasome	Proteasome subunit beta type 6,9, putative. Multicatalytic endopeptidase complex, proteasome precursor, beta subunit		*Ricinus communis, Arabidopsis thaliana*

Gene	Description	Example/Type	Species
Ring finger protein, putative	Protein binding / ubiquitin-protein ligase/zinc ion binding	RING-finger (**Really Interesting New Gene**) domain, a specialised type of Zn-finger of 40 to 60 residues that binds two atoms of zinc; defined by the 'cross-brace' motif C-X2-C-X(9-39)-C-X(1-3)- H-X(2-3)-(N/C/H)-X2-C-X(4-48) C-X2-C; probably involved in mediating **protein-protein interactions**; identified in a protein with a wide range of functions such as viral replication, signal transduction and development	*Ricinus communis, Arabidopsis thaliana*
	Putative senescence-associated protein		*Cupressus sempervirens*; (common cypress) *Capsicum annuum; Pisum sativum*
Salt stress?	Senescence-associated protein; cytochrome P450 TBP-like protein (TATA binding **protein**-**like**); 26S ribosomal RNA gene		*Phytolacca dioica, Picea abies, Lilium longiflorum*
	Ubiquitin-protein ligase/zinc ion binding		*Arabidopsis thaliana*
Zinc finger at the C-terminus of An1, a ubiquitin-like protein	AN1-like zinc finger	Putative CCCH-type zinc finger protein. AN1-type; Zinc finger, A20-type	*Medicago truncatula*
	Peptidase family M41. ATP-dependent peptidase activity/ metallopeptidase activity/proteolysis and peptidolysis/protein catabolism		
Peptidase family M20/M25/M40.- This family includes a range of zinc metallopeptidases belonging to several families in the peptidase classification. Family M20 is glutamate carboxypeptidases. Peptidase family M25 contains X-His dipeptidases. Metal-dependent amidase aminoacylase carboxy peptidase	ILL5; metallopeptidase (ILL5) mRNA, complete cds		*Arabidopsis thaliana*
WLM domain. - This is a predicted metallopeptidase domain called WLM (Wss1p-like metalloproteases)	Genetic evidence implicates the WLM family in **de-SUMOylation**. Novel predicted peptidases with a potential role in the **ubiquitin** signalling pathway	Zinc metalloproteinase-like	
Clathrin adaptor complex small chain			*Cicer arietinum*
Ubiquitin-conjugating enzyme, E2			*Medicago truncatula*

Gene	Description	Example/Type	Species
mRNA for polyubiquitin	Ubiquitin-mediated proteolysis is part of the regulated turnover of proteins required for controlling cell cycle progression		*Plantago major*
Function: Kinases, phosphatases, G proteins, ATP, AMP			
Nucleoside diphosphate kinases (NDP kinases, NDPks)	NDP kinases, responsible for the synthesis of nucleoside triphosphates (NTPs), are involved in numerous regulatory processes associated with proliferation, development and differentiation	cDNA clone S02D21 5' similar to nucleoside diphosphate kinase	*Chenopodium quinoa; M. crystallinum*
The NPH3 gene	NPH3 is a member of a large protein family, apparently specific to higher plants, and may function as an adapter or scaffold protein to bring together the enzymatic components of a **NPH1-activated phosphorelay**	Phototropism of *Arabi- dopsis thaliana* seedlings in response to a blue light source is initiated by nonphototropic hypocotyl 1 (NPH1)	Unnamed protein product [*Vitis vinifera*]
Small GTPases	ARF GTPase activator/zinc ion binding		*Arabidopsis thaliana*
	AMP dependent ligase, putative. **AMP-binding** protein	**SNF1-related protein kinases** in higher plants are likely to be involved in the response of plant cells to environmental and/or nutritional stress	*Ricinus communis*
Ras-like GTPase. - Ras-like GTPase superfamily	Putative RAS-related GTP-binding protein		
	ATP binding / kinase / protein kinase/ protein serine/ threonine kinase		*Arabidopsis thaliana*
Ankyrin-kinase			*Medicago truncatula*
Cyclin-dependent kinases regulatory subunit			*Glycine max*
ATP synthase			*Spinacia oleracea*
mRNA for subunit A of the V-type ATPase	Vacuolar-type H+-ATPase subunit A		*Beta vulgaris*
ATPase, subunit C of V-type ATPase	ATP-syntC, ATP synthase subunit C		*Beta vulgaris* subsp. vulgaris
ATPase beta subunit	It uses a proton gradient to drive ATP synthesis and hydrolyses ATP to build the proton gradient. The extrinisic membrane domain, F1, is composed of **alpha, beta, gamma, delta and epsilon** subunits with a stoichiometry of 3:3:1:1:1. The beta subunit of ATP synthase is catalytic		*Nicotiana sylvestris; Gossypium hirsutum*
MAP kinase, PKc-like	Protein kinases, catalytic domain// The protein kinase superfamily is mainly composed of the catalytic domains of serine/threonine-specific and tyrosine-specific protein kinases		*Gossypium hirsutum*
Receptor protein kinase-like			*Arabidopsis thaliana*

Gene	Description	Example/Type	Species
Forisomes are Ca(2+)-dependent contractile protein			
Endonuclease/exonuclease/phosphatase family protein / calcium-binding EF hand family protein	This family includes: AP endonuclease proteins EC:4.2.99.18, DNase I proteins EC:3.1.21.1, Synaptojanin and inositol-1,4,5-trisphosphate phosphatase EC:3.1.3.56, Sphingomyelinase EC:3.1.4.12 and **Nocturnin**	**Apurinic/apyrimidinic (AP) endonuclease** is an enzyme that is involved in the DNA base excision repair pathway (BER)	*Arabidopsis thaliana*
Pto kinase interactor 1	**Mitogen-activated protein kinase (MAPK) kinase kinase (MAPKKK) subfamily**, catalytic (c) domain	The MAPKKK subfamily is part of a larger superfamily that includes the catalytic domains of other protein STKs, protein tyrosine kinases, RIO kinases, aminoglycoside phospho transferase, choline kinase and phospho inositide 3-kinase	*Lycopersicon esculentum*
Function: RNA silencing			
Piwi-like: PIWI domain. Domain found in proteins involved in RNA silencing	RNA silencing refers to a group of related gene-silencing mechanisms mediated by short RNA molecules, including siRNAs, miRNAs and heterochromatin-related guide RNAs. The central component of the **RNA-induced silencing complex (RISC)** and related complexes is **Argonaute.** The PIWI domain is the C-terminal portion of Argonaute and consists of two subdomains, one of which provides the 5' anchoring of the guide RNA and the other the catalytic site for silencing	AGO1-1; Scarlet Runner Bean Suspensor Region TriplEx2; cDNA 5' similar to Argonaute protein, mRNA sequence	*Nicotiana benthamia na; Phaseolus coccineus*
S-adenosyl-L-homocysteine hydrolase	Mutations of the *HOMOLOGY-DEPENDENT GENE SILENCING1* (*HOG1*) locus relieve transcriptional gene silencing and methylation-dependent HDG silencing and result in genome-wide demethylation		***Beta vulgaris***
Function: Transcription Factors			
Homeodomain transcription factor (ATHB-6)	BEL1-like homeobox 2 protein (BLH2) (At4g36870)		*Arabidopsis thaliana*
HTH_XRE. - Helix-turn-helix XRE-family like proteins	Prokaryotic DNA binding proteins belonging to the xenobiotic response element family of transcriptional regulators		**Developing root *Beta vulgaris* cDNA clone**
CONSTANS interacting protein 2b (CIP2b)	**Histone-like transcription factor** (CBF/NF-Y) and archaeal histone: This family includes archaebacterial histones and histone-like transcription factors from eukaryotes		*Lycopersicon esculentum; Medicago truncatula*
SNF2-related domain-containing protein	Chromatin remodelling; ATP binding / DNA binding / helicase		*Dictyostelium discoideum* AX4
Function: Secondary metabolites, terpenes, phenylpropanoids, volatiles			
Geranylgeranyl hydrogenase			*Mesembryanthemum crystallinum*

Gene	Description	Example/Type	Species
Geranylgeranyl pyrophosphate synthase-related protein	This CD includes all-trans (E)-isoprenyl diphosphate synthases which synthesise various chain length (C10, C15, C20, C25, C30, C35, C40, C45 and C50) linear isoprenyl diphosphates from precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP)		
1-deoxy-D-xylulose 5-phosphate reductoisomerase precursor			*Hevea brasiliensis, Oryza sativa*
Chorismate synthase (CS2), mRNA	The chorismate synthase (CS) catalyses the seventh step in this pathway, the conversion of 5-enolpyruvylshikimate-3-phosphate to chorismate. Ver: MPMI, March 2009, Volume 22, Number 3 Pages 311-320	Plant processes resulting from primary or secondary metabolism have been hypothesised to contribute to defence against microbial attack. Barley chorismate synthase (*HvCS*), anthranilate synthase α subunit 2 (*HvASa2*) and chorismate mutase 1 (*HvCM1*) occupy pivotal branch points downstream of the shikimate pathway leading to the synthesis of aromatic amino acids. Here, we provide functional evidence that these genes contribute to penetration resistance to *Blumeria graminis* f. sp. *hordei*, the causal agent of powdery mildew disease	*Populus trichocarpa*
Cytochrome P450 (CYP98A27); mRNA, p-coumaryl-CoA 3'-hydroxylase	Impact on lignin deposition and soluble secondary metabolism		*Populus trichocarpa* [*Populus alba* x *Populus grandidentata*]

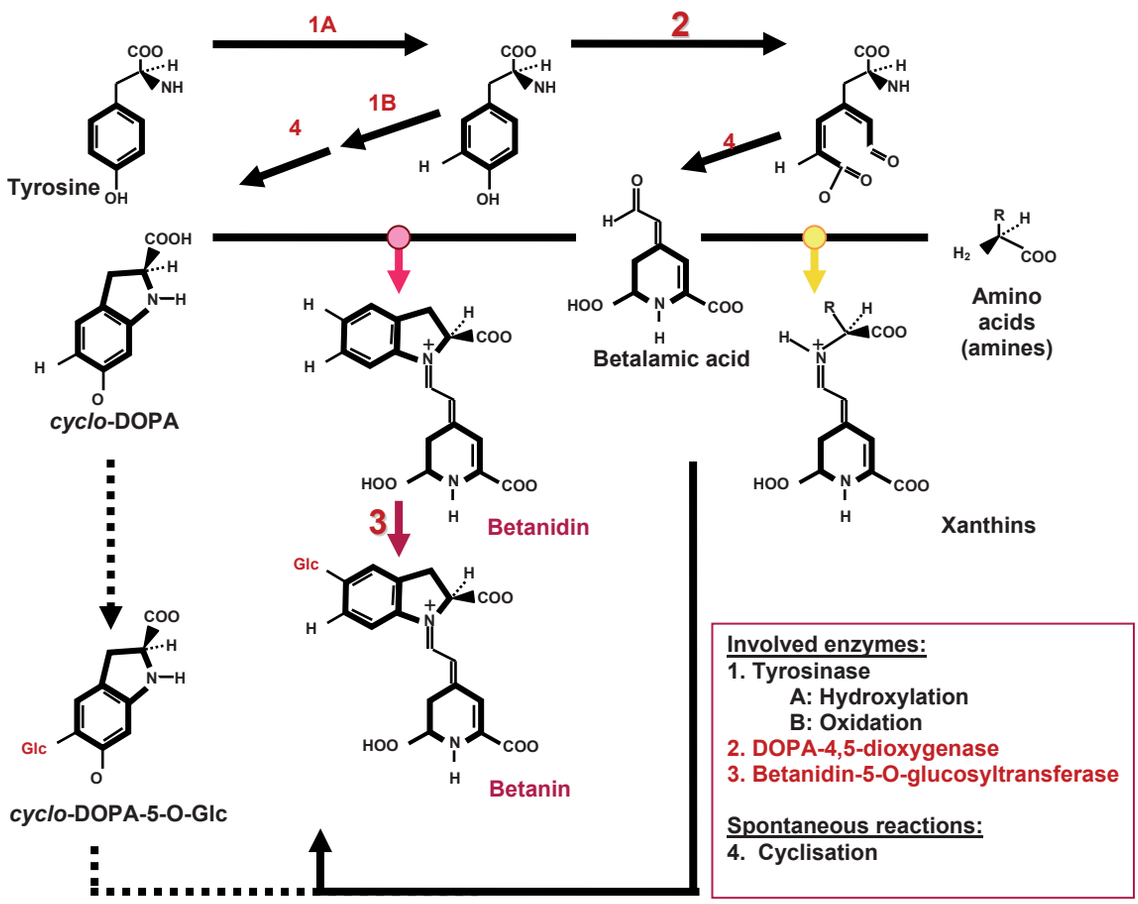

Figure 1.5.1. Proposed scheme for betalain byosynthesis in the Cariophylalles order in which the *Amaranthaceae* are included.

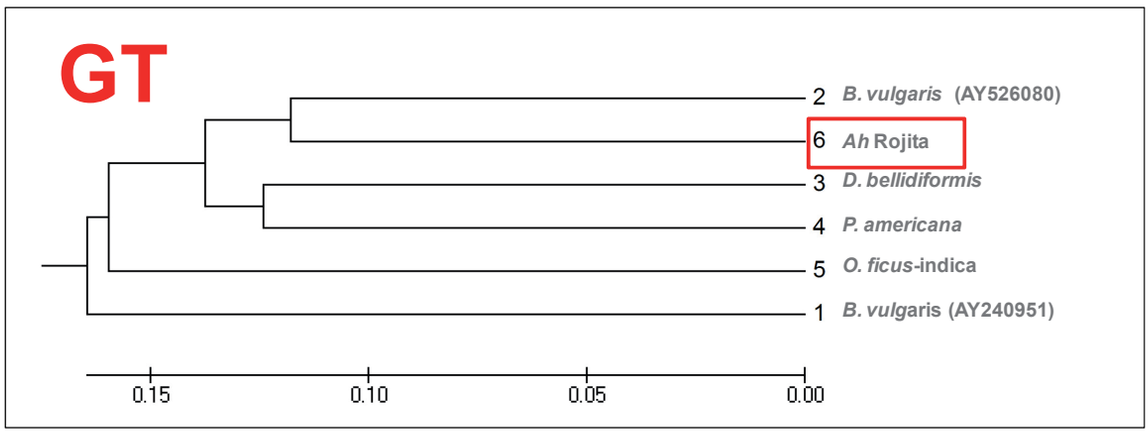

Figure 1.5.2. Phylogenetic analysis of the partial cDNA sequence of a betanidin 5-O-glucosyl transferase cDNA sequence isolated from leaves of *A. hypochondriacus* cv. Rojita (*Ah* Rojita). The partial cDNA sequence was obtained from an experimental PCR approach based on the use of degenerate primers designed on the basis of conserved regions found in similar genes in related species such as: *Beta vulgaris*, *Dorotheanthus bellidiformis*/*Mesembryanthemum criniflorum*, *Portulaca americana* and *Opuntia ficus-indica*.

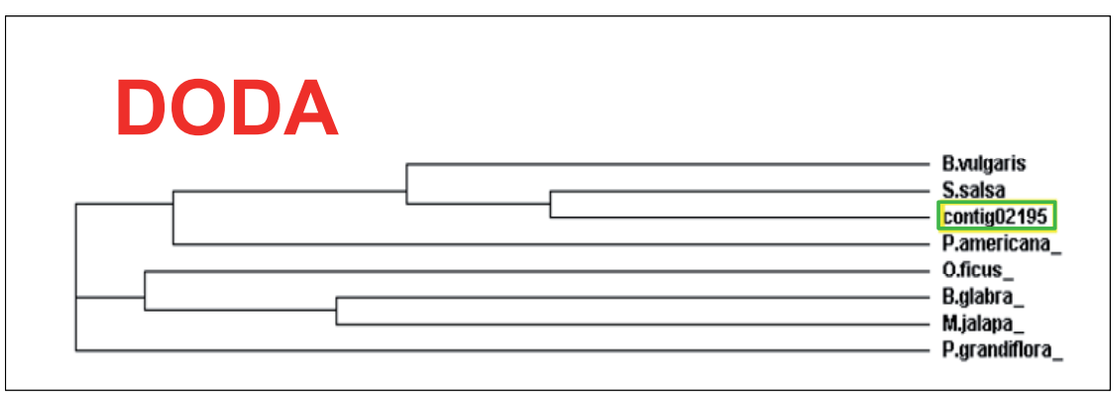

Figure 1.5.3. Phylogenetic analysis of the partial cDNA sequence of DOPA-4, 5-dioxygense (DODA) obtained from the partial transcriptome sequencing of *A. hypochondriacus* cv. Revancha (contig 02195) as compared to sequences of known DODA genes in related species such as: *Beta vulgaris*, *Suaeda salsa*, *Portulaca americana*, *Opuntia ficus-indica*, *Beta vulgaris* var. glabra, *Mirabilis jalapa* and *Portulaca grandiflora*.

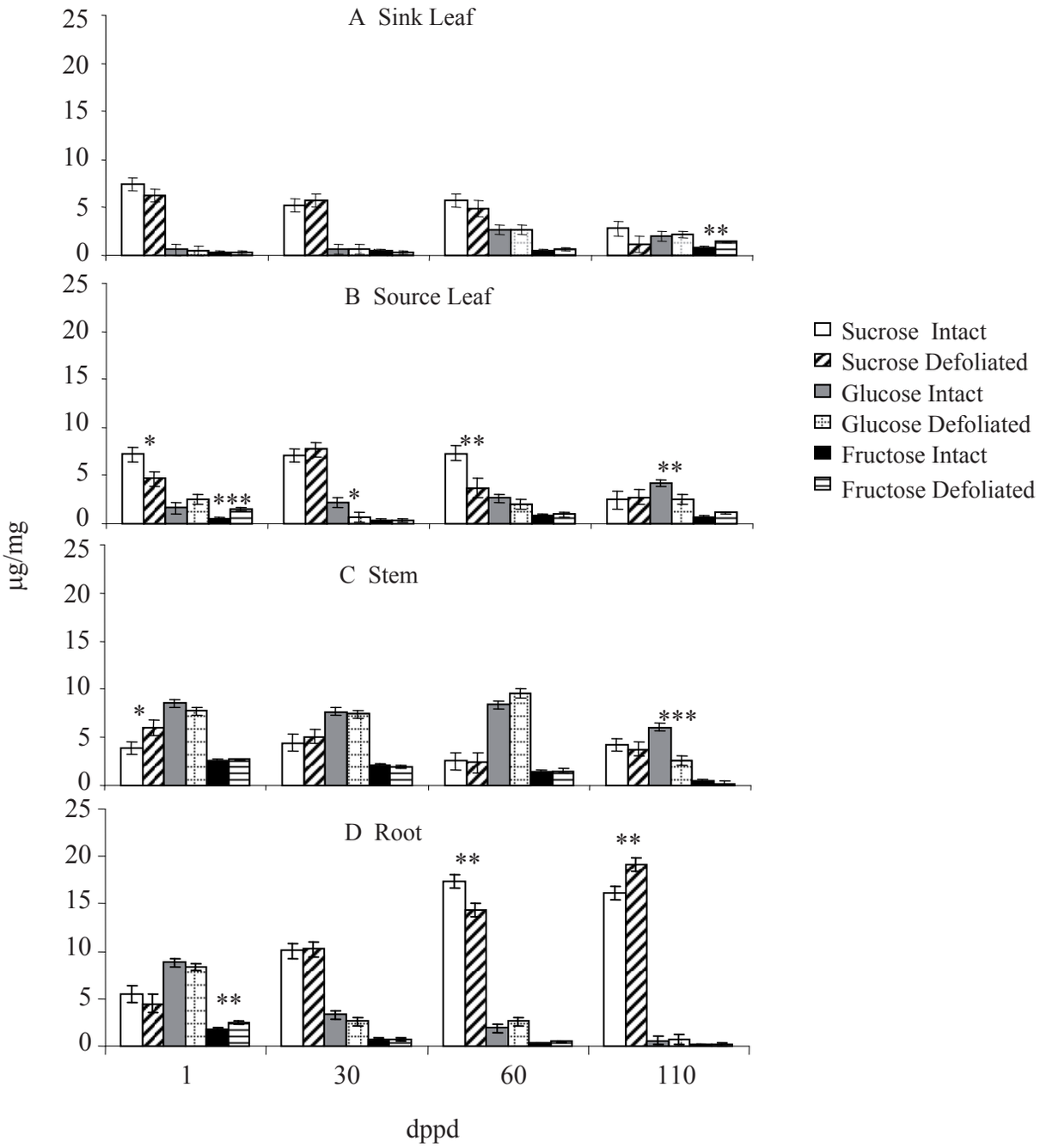

Figure 1.5.4. Time course variation in sucrose, glucose and fructose levels measured at different days post partial defoliation (dppd) in **(A)** sink leaves, **(B)** source leaves, **(C)** stems and **(D)** roots of intact and partially defoliated *A. cruentus* plants. Data were analysed using one-way *ANOVA* for the treatment (partial defoliation) × days post treatment × tissue interaction. *, $P < 0.05$; **, $P < 0.01$ and ***, $P < 0.001$.

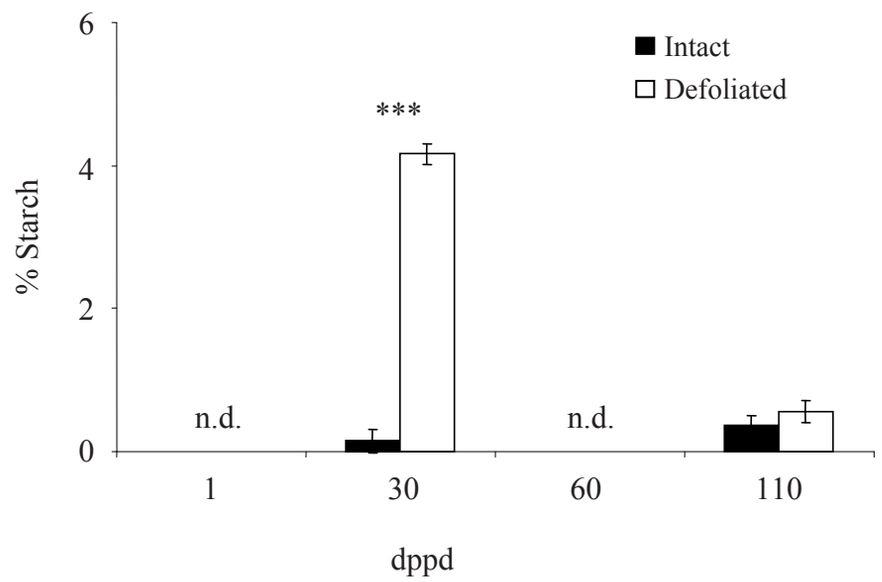

Figure 1.5.5. Time course variation in starch levels measured at different days post partial defoliation (dppd) in stems of intact and partially defoliated *A. cruentus* plants. Starch was almost 30 times higher in stems of defoliated plants 30 dppd ($P < 0.0001$). Data were analysed using one way ANOVA.

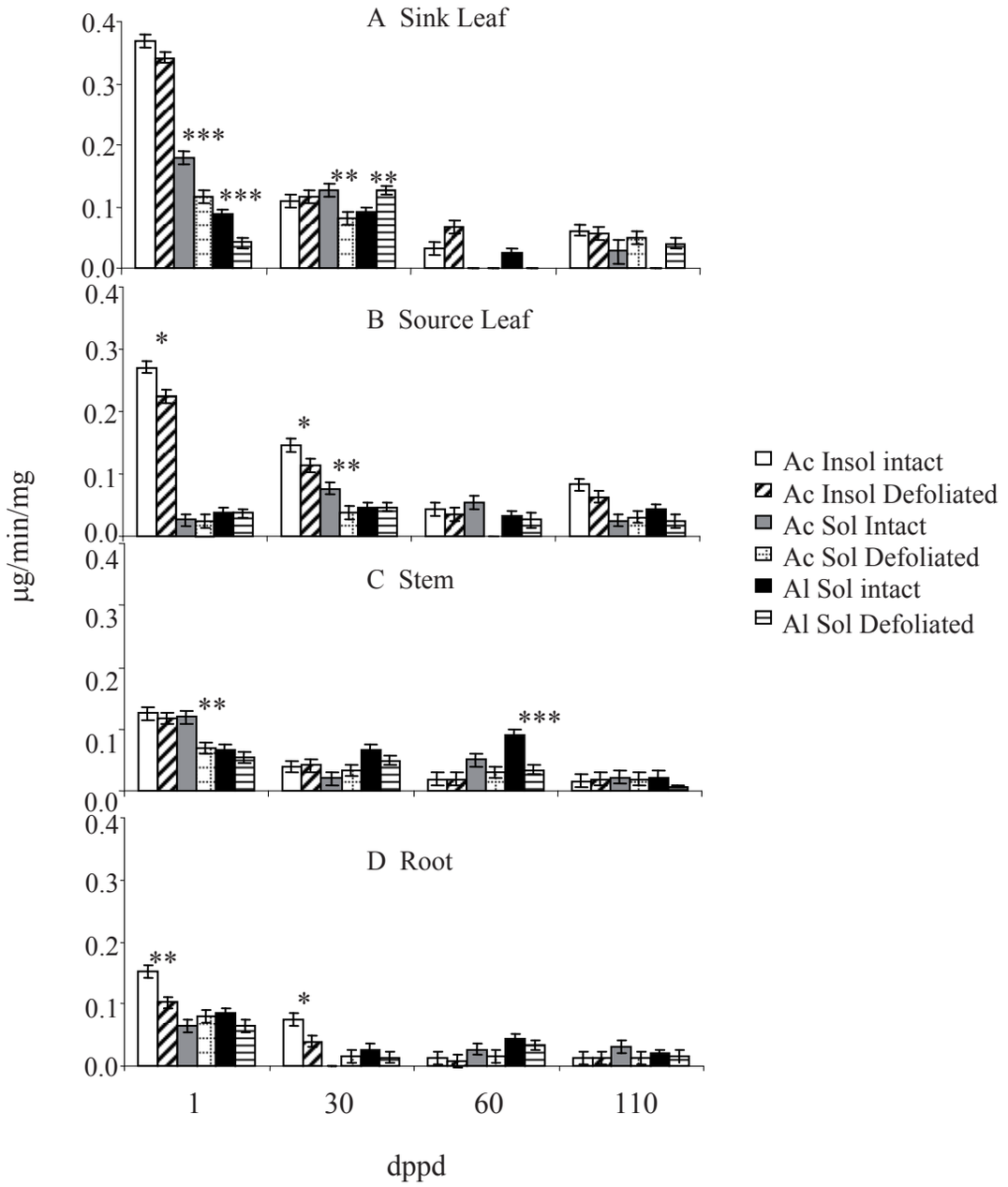

Figure 1.5.6. Time course variation in the enzymatic activity of insoluble acid invertases, soluble acid invertases and soluble alkaline invertases measured at different days post partial defoliation (dppd) in **(A)** sink leaves, **(B)** source leaves, **(C)** stems and **(D)** roots of intact and partially defoliated *A. cruentus* plants. Data were analysed using one-way *ANOVA* for the treatment (partial defoliation) × days post treatment × tissue interaction. *, $P < 0.05$; **, $P < 0.01$ and ***, $P < 0.001$.

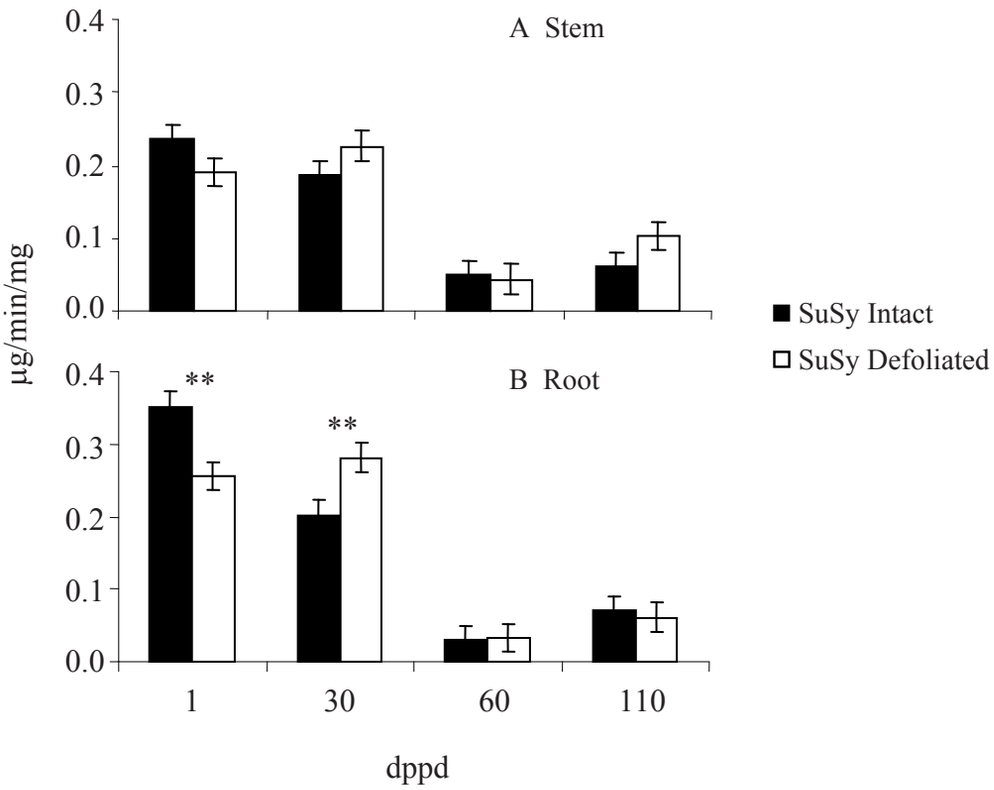

Figure 1.5.7. Time course variation in the sucrolytic activity of Sucrose Synthase measured at different days post partial defoliation (dppd) in **(A)** stems and **(B)** roots of intact and partially defoliated *A. cruentus* plants. Data were analysed using one-way *ANOVA* for the treatment (partial defoliation) × days post treatment × tissue interaction. *, $P < 0.05$; **, $P < 0.01$ and ***, $P < 0.001$.

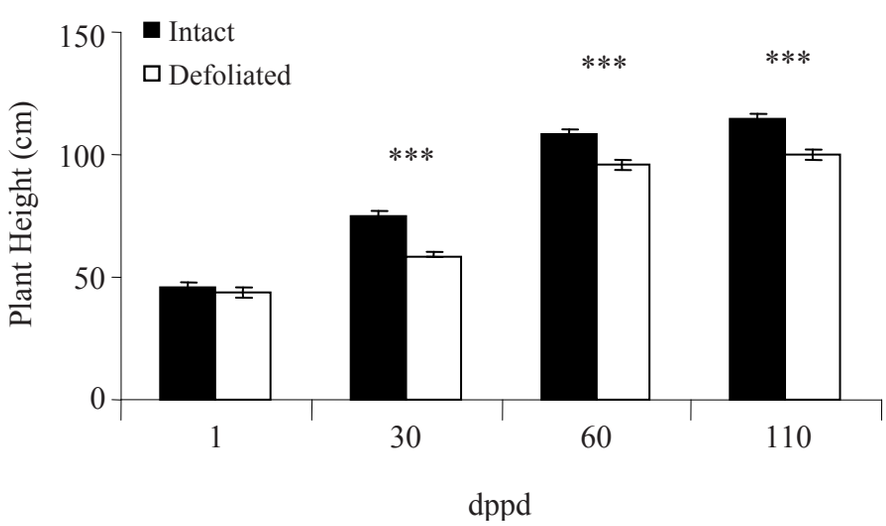

Figure 1.5.8. Time course variation in plant height (in cm) measured at different days post partial defoliation (dppd) in intact and partially defoliated *A. cruentus* plants. Defoliated plants were significantly smaller ($P < 0.001$) than intact plants after 30 dppd.

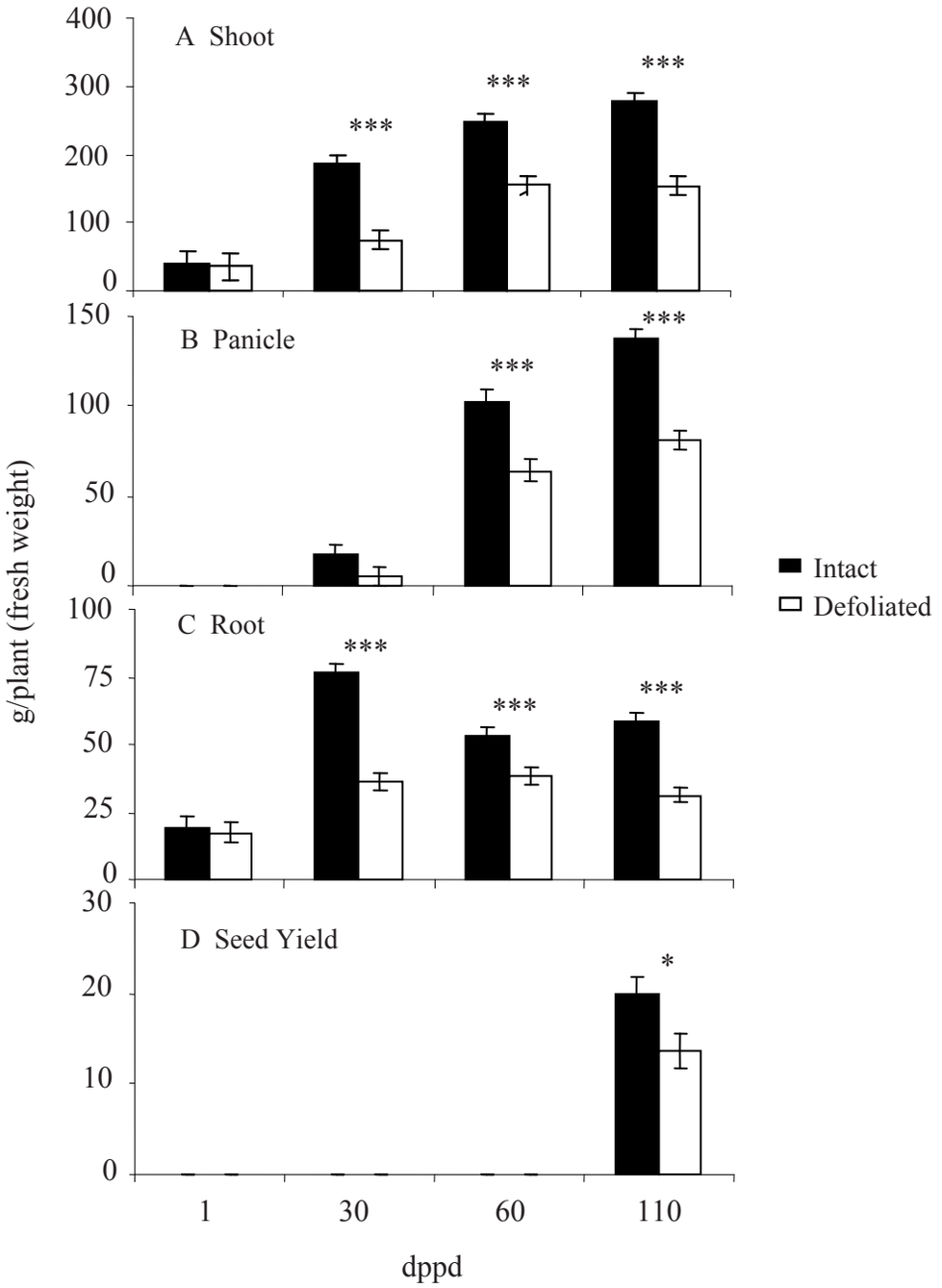

Figure 1.5.9. Fitness traits difference between intact and defoliated *A. cruentus* plants. Defoliated plants gained less weight during development, **(A)** to **(C)**, and produced less seed, **(D)**, than intact plants. Data were analysed by one way ANOVA; *, $P < 0.05$; **, $P < 0.01$, and ***, $P < 0.001$.

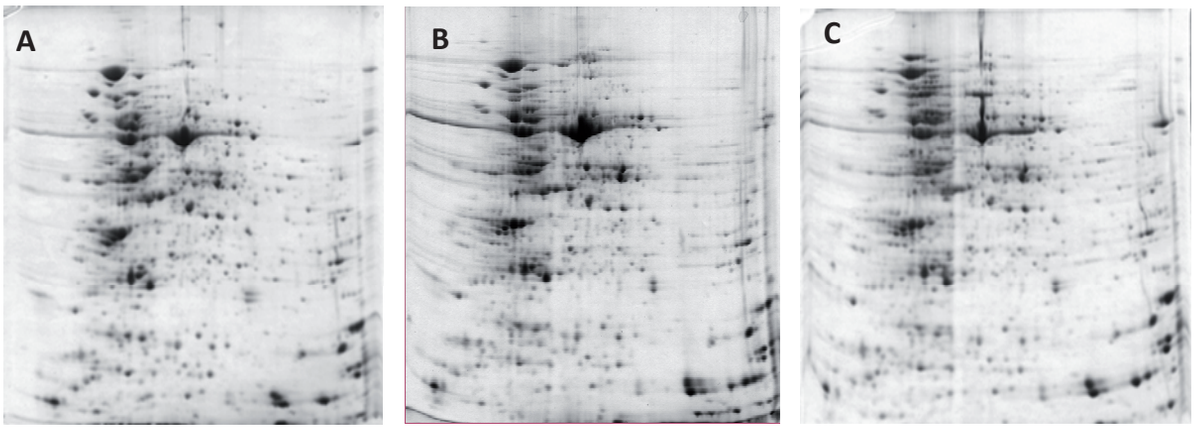

Figure 1.5.10. Proteomic 2-D maps of total leaf proteins extracted from of grain amaranth (*A. hypochondriacus*) plants subjected to insect herbivory by larvae of *Spodoptera exigua*. The images represent representative 2-D patterns obtained from intact leaves **(A)**, damaged leaves (local response, **B)** and undamaged, distal leaves (systemic response, **C)**.

1.6 Drought and salinity resistance

1.6.1 Objective

The goal of this research was to provide an overview of the differential genes and proteins expressed under abiotic stress. 1. For differential gene expression the experimental approach used was the Suppressive Subtractive Hybridisation (SSH). 2. For differential protein expression the experimental approach used was the 2-DE and MS analysis, the proteomics approach.

1.6.2 Methodology

Amaranth plants were grown and seedlings were subjected to water deficit or salt stress. After 7 days of stress, the roots and leaves of plants were collected and frozen under liquid nitrogen and kept at -80°C, until used.

1. Differential gene expression

Isolation of total RNA and mRNA. The frozen leaves were ground with a mortar and pestle to get a fine powder. Total RNA was isolated from frozen leaf tissue using RNeasy Mini Kit system (Qiagen, GMBH, Hilden) according to manufacturer´s instructions. RNA quantity and its quality was checked by Agarose electrophoresis gel.

Suppression subtractive hybridisation (SSH). cDNA was synthetised using the Super Script™ II Reverse Transcriptase (Invitrogen) and the SMART™ PCR cDNA Synthesis Kit (Clontech) according to the manufacturer´s instructions. Starting with 1.5 µg of total RNA from the tissues being compared (leaves of amaranth in stress and control), cDNA was purified on columns of Chroma Spin-1000 DEPC (Clontech). SSH was carried out using the PCR-Select™ cDNA Subtraction Kit (Clontech) according to the manufacturer´s instructions. Leaf tissue of amaranth in stress and control were processed simultaneously to reduce false positives.

Sequence analyses. Colonies were randomly picked from each plated subtractive cDNA library for restriction analysis. About 130 fragments belonging to genes were sequenced. The edited sequences were compared to the GenBank database using BLASTX and BLASTN (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Detection of gene expression level for semiquantitative RT-PCR. The primer used for RT-PCR was designed from the cDNA sequences of the genes mt2a, C3HC4-type ring zinc finger, kinase, S-Adenosyl methionine (SAM), CaM and STSA were found in the subtractive library. The PCR products were checked by 1.8% agarose gel in 1X TAE and stained with ethidium bromide. The expression level of genes in each amaranth sample was checked and calculated based on the intensity of the band by Quantity One™ v 4.5.0 (BIO-RAD). *Actin* was used as an internal standard.

Northern blot. The RNA was transferred to Hynbond N + membrane by capillary action. 25 ng of each probes to be evaluated was denatured and marked with dCTP32, the marking was done at 37°C for 1hr.

2. Differential protein expression

Protein extraction. Total soluble root and leaf proteins were extracted according to the method of Sarvanan and Rose (2004) with standardised conditions developed in our lab (Huerta-Ocampo *et al.*, 2009).

Two-dimensional gel electrophoresis. For isoelectric focusing (IEF), 13-cm IPG linear gradient

stripos (pH 4-7) were rehydrated at room temperature for 14-16 hr. Focusing was carried out in an Ettan IPGphor system (GE Helathcare) at a constant 50 mA per strip at 20˚C. Running was carried out according to the manufacturer's instructions. The strips were equilibrated for 15 min in an equilibration buffer and second dimension was run in a 13% gel. Gels were stained and digitalised. Data analysis of differentially expressed proteins was performed by One Way ANOVA (Statgraphics Plus v5.0).

LC/ESI-MS/MS). The protein spots were carefully excised from the stained 2-DE gels and in gel digested with trypsin (Promega). Mass spectrometric analysis was carried out on a 3200Q Trap hybrid tandem mass spectrometer (Applied Biosystems/MDS Sciex) equipped with a nano electrospray ion source. The instrument was coupled online to a nanoAcquity Ultra Performance LC system (Waters).

Data interpretation. Protein identification was performed on the Ms/Ms spectra datasets using the MASCOT search algorithm. Searches were conducted using the Viridiplantae subset of the NCBI.

1.6.3 End results

The analysis of the amaranth leaves transcriptome showed several differential gene expressions (Figure 1.6.1.A). Clones were grouped according to its function as: metabolism, heat shock proteins, transcription factors, signal transduction, defence and photosynthesis. Genes with no homology and genes whose function is unknown were found.

Among transcription factors two studied targets were DOF1 and MIF1, both are related to the cell growth and differentiation (Huerta-Ocampo *et al.*, 2010). Another transcription factor, the ZnFg, was found up-regulated (Figure 1.6.1.B); the complete sequence of this gene has proved to have a different homology to those reported (Aguilar-Hernández *et al.*, 2010).

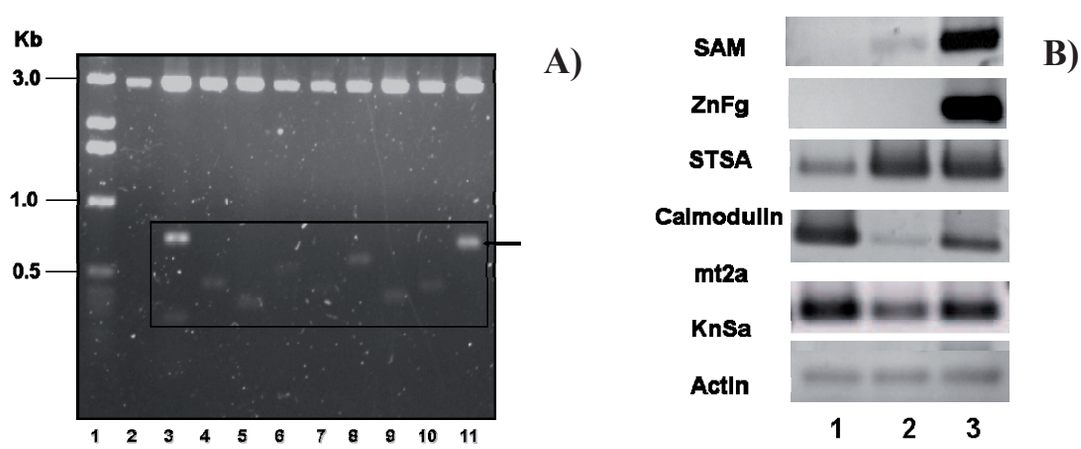

Figure 1.6.1. A) SSH libraries show different differential gene expression. B) Northern Blot analysis confirms the up-and down- regulation of some of those genes.

Also, different kinases were found as part of signal transduction cascade; these results have opened new questions about the importance of phosphorilation pathways in response to abiotic stress.

At protein level the finding was interesting that a group of heath shock proteins belonging to the small chaperonin family were up-regulated during abiotic stress (Table 1.6.1). This together with the fact that RUBISCO degradation was not found shows the importance of this family of proteins in protein stabilisation during stress.

Table 1.6.1. Identification of drought-responsive proteins in *Amaranthus hypochondriacus* L. roots by LC/ESI_MS/MS analysis.

Spot no.[a]	Protein change[‡]	Peptides matched / Secuence coverage	Mascot Score [b]	Protein name	Accession no.[c]	Exp.Mass (kDa) / pI	Theor.Mass (kDa) / pI	Source
1 *	U	2 / 11%	109	Chaperonin 21 precursor	gi|7331143	22.6 / 5.43	26.5 / 6.85	*L. esculentum*
2 *	D	10 / 35%	401	S-adenosylmethionine synthetase 2	gi|127046	43.4 / 5.66	43.2 /5.57	*Dianthus caryophyllus*
3 *	D	15 / 53%	506	S-adenosylmethionine synthetase	gi|71000465	43.1 / 5.97	42.9/ 5.60	*Beta vulgaris*
4 *	U	1 / 10%	45	Glycine-rich RNA-binding protein 1	gi|6911142	15.4 / 6.54	14.1/8.71	*Catharanthus roseus*
5 *	U	1 / 9%	52	Glycine-rich RNA-binding protein 2	gi|6911146	18.8 / 6.33	16.2/7.82	*Catharanthus roseus*
6 *	U	3 / 21%	122	Cytosolic class I HSP 2B	gi|37704425	16.8 / 5.65	15.3/5.82	*Nicotiana tabacum*
7 *	U	3 / 21%	93	17.5 kDa Class I HSP	gi|38639431	16.2 / 5.64	17.5/5.31	*Carica papaya*
8 *	U	2 / 9%	95	Low Molecular Weight HSP	gi|15913894	19.6 / 6.04	18.3/6.34	*Gossypium hirsutum*
10 *	U	4 / 24%	138	Small Heat Shock Protein	gi|16930753	17.5 / 5.47	17.9/5.82	*Retama raetam*
11 *	D	3 / 10%	48	Isoflavone reductase-like protein	gi|18410820	32.5 / 5.46	33.7/5.66	*Arabidopsis thaliana*
12 *	D	4 / 16%	92	Adenosine kinase 1	gi|15232763	38.5 / 5.11	37.8/5.29	*Arabidopsis thaliana*
13 *	D	3 / 9%	119	Caffeic acid 3-O-methyltransferase	gi|3176967	36.8 / 6.14	38.6/ 5.70	*M. crystallinum*
16 *	U	2 / 16%	50	Heat Shock Protein 17.6 kDa	gi|15218934	16.8 /6.11	17.7/6.85	*Arabidopsis thaliana*
19 *	U	1 / 8%	56	Superoxide dismutase [Cu-Zn]	gi|3334334	15.6 / 5.41	15.2/5.46	*M. crystallinum*
20 *	U	6 / 38%	153	Nucleoside diphosphate kinase 1	gi|400404	12.6 / 6.55	16.3/6.42	*Spinacia oleracea*

a = The numbers corresponds to the 2-DE gel showed in figure 1. *b*= Scores > 44 indicate identity or extensive homology (P <0.05) c= Accession numbers are from NCBI Entrez database *= The treatment effect is significant, P < 0.05, ‡ = D, Upregulated proteins; D, downregulated proteins. HSP, Heath Shock Protein.

Because the accumulation of reactive oxygen species (ROS) as a result of various environmental stresses is a major cause of loss of crop productivity, superoxide dismutases (SODs) are described to be the first line of defence against highly toxic superoxide radicals by rapidly converting superoxide to hydrogen peroxide (H_2O_2) and molecular oxygen. From our results (Table 1.6.1) we have found one Cu-Zn superoxide dismutase; this enzyme was cloned (Figure 1.6.2A) and overexpressed in recombinant systems (Figure 1.6.2B) and

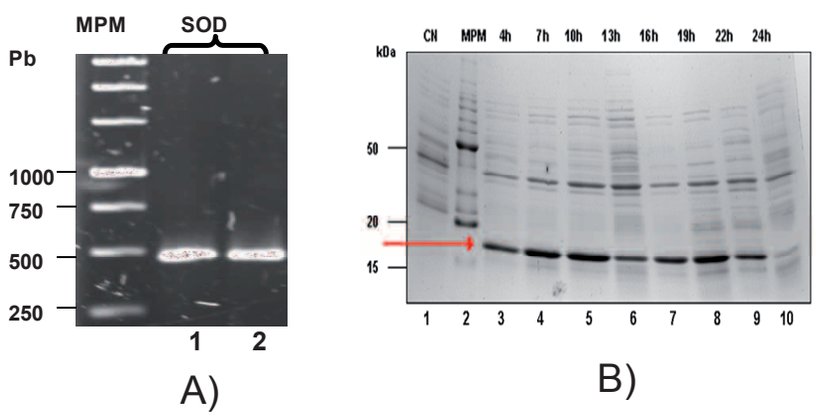

Figure 1.6.2. A) Amplification of full open reading frame (ORF) for the Cu,Zn Superoxide Dismutase. B) Expression of recombinant protein in *Escherichia coli*. The red arrow indicates the 17 kDa SOD protein expressed at different culture times.

characterised. Results of this part of work are in the process of manuscript preparation (Cruz-Ortega *et al.*, 2010).

1.6.4 Impact of the project

We reached the objective of this research area. Now we have results and the first genes and proteins in response to the salt/water stress in amaranth. These results have opened new questions about and new insights into the natural tolerance to stress in plants. These results contribute substantially to "the state of the art" because we have amplified the use of proteomics in the elucidation of amaranth drought/salinity stress.

The results of this work and the presentations at national meetings have attracted the interest of industry and agriculture. In the present government (2009-2015) of the San Luis Potosi State, Mexico, amaranth is taking an important place in the development of sustainable agriculture in the arid zones of the state, as well in the preparation of children's breakfast for the DIF (Family Integral Development).

1.7 Crop competetiveness and weed control

1.7.1 Objective

To identify genotypes of amaranth with a high degree of competitiveness with weeds and develop mechanical weed control strategies to be used in combination with a selection of competitive species.

1.7.2 Methodology

Mechanical weed control

Amaranth is traditionally sown at wide row distances. This allows for the use of interrow cultivators or for other types of mechanical weed control procedures. Studying mechanical weed control practices was not an objective of the project, but because no herbicides are registered for use in amaranth in any of the partner countries manual and mechanical weed control were the only methods available in the field experiments to prevent yield losses from weed competition. Hence, an experiment with 3 amaranth accessions was set up in Argentina and Spain. In Argentina an interrow cultivator was used, while the experiment in Spain studied the effect and selectivity of weed harrowing.

Competitiveness of amaranth accessions

The competiveness of three amaranth accessions (*A. hypochondriacus* (cv. San Antonio), *A. cruentus* (cv. Don Armando and cv. Don Juan) against *Echinochloa crus-galli*, a grass weed species, and *Solanum nigrum*, a broadleaved weed species, was studied in two response surface experiments. The data were analysed using a discrete hyperbolic competition model.

Crop competiveness against weeds was also studied in a field trial in which crop density was varied from 100.000 to 400.000 plants/ha but maintaining the same row distance.

Preliminary identification of allelopathic potential of amaranth accessions

Standardised laboratory Petri dish assays were conducted to assess the allelopathic potential of four amaranth accessions with varying content of secondary metabolites: Don Leon (high phenolic acid content/high flavonoid content), Don Armando (low phenolic acid content/ high flavonoid content), San Antonio (high phenolic acid content/low flavonoid content) and Ravancha morfotipo Mercado (low phenolic acid content/low flavonoid content). The potential effect on seed germination and early seedling growth was tested using 3 weed species: *Veronica agrestis*, *Poa annua* and *Lolium perenne*.

Survival and persistence in soil of amaranth seeds

Seeds of 3 amaranth species (*Amaranthus cruentus* (cv. Don Guiem), *A. hypochondriacus* (cv. Nutrisol) and *A. mantegazzianus* (cv. Don Juan)) were buried at 4 depths (2, 5, 10 and 25 cm) in Argentina, Spain and Denmark. The seeds were excavated one year after burial, and germination was examined under controlled conditions. Survival of seeds on the soil surface was also studied in the field for a period of 1 month following harvest.

Identifying potential herbicides

The selectivity of a range of herbicides applied pre-, early post- and late post-emergence was

studied in pot trials in the glasshouse (Figure 1.7.1). The impact of sowing depth on crop selectivity was also studied. Promising candidates were subsequently tested in a small-plot field experiment.

Developing weed control strategies
A guide on weed control strategies for dissemination to end-users (advisors and farmers) summarising the outputs of the project was produced.

1.7.3 End results
Not surprisingly interrow cultivation was an effective and selective control option, but the capacity is low. The capacity of weed harrowing is much higher, but in contrast to interrow cultivation the crop will also be affected (de Troiani *et al.*, 2008). The experiment in Spain revaled that although the amaranth crop was visually affected by weed harrowing it recovered quickly and the conclusion of the experiment was that amaranth seems to tolerate weed harrowing quite well.

The statistical analyses of of the competion experiments revealed differences in competitiveness between the 3 accessions. Don Juan was less competitive than Don Armando and San Antonio suggesting that the varieties differ in their ability to suppress weeds and that this is an agronomic trait that should be considered when selecting an amaranth species/variety (Mathiassen and Kudsk, 2010).

Figure 1.7.1. Photo from the glasshouse of the competition experiment with *E. crus galli*.

In the field trial with different crop densities it was observed that weed infestation was lower at a density of 400,000 plants/ha with 47% of surface covered by weeds, compared to 67 and 66% at 100,000 and 200.000 plants/ha. Increasing crop density will minimise the weed problems particularly within the row. To suppress the weed population between the rows the row distance would have to be reduced.

The presence of amaranth seeds did not affect weed seed germination; however, root and especially shoot lengths of the small-seeded species *V. agrestis* and *P. annua* were reduced by up to 50% while the growth of the large-seeded species *L. perenne* was unaffected by the presence of amaranth. A number of flavonoids and phenolic acids were identified and quantified by LCMSMS using activity-guided fractionation of filter paper from the Petri dishes (Figure 1.7.2). The most abundant phenolic acids were ferulic acid and vanillic acid. No significant differences in concentrations of the phenolic acids were found between varieties (Mathiassen *et al.*, 2008; 2009).

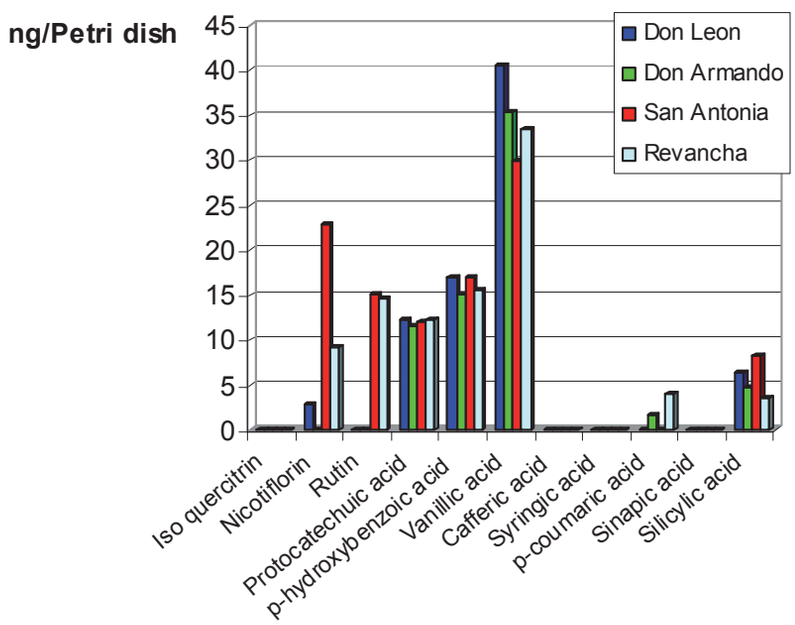

Figure 1.7.2. Content of flavonoids and phenolic acids in filter papers from Petri dishes with germinating seeds of the different amaranth species/varities. The content of ferulic acid was from 175-200 ng/Petri dish.

Seed survival of amaranth seeds was very low, i.e. weed problems caused by volunteer amaranth plants can be assumed to be insignificant, even though seed loss prior to harvest will be significant with the available varieties. Reducing seed shattering prior to harvest should be an objective of future breeding programmes.

Clomazone was the only pre-emergence treatment that did not affect either seed germination or plant growth. The selectivity was higher when the seeds were covered by 1.5 compared to 0.5 cm soil. Clomazone was also tolerated by amaranth early post-emergence (up to the 2-leaf stage). The screenings at later growth stages showed that phenmedipham, clopyralid and triflusulfuron can be used for broadleaf weed control and fenoxaprop-P and fluazifop-P-butyl for control of grass weeds in amaranth. In contrast low doses of fluroxypyr, ioxynil+bromoxynil, tribenuron, thifensulfuron, amidosulfuron, flupyrsulfuron, iodosulfuron and foramsulfuron caused severe plant injury. The screenings did not indicate any differences in herbicide tolerance of *A.*

cruentus, *A. caudatus* and *A. mantegazzianum*. The small-plot field experiment confirmed the results obtained in the pot experiments (Kudsk and Mathiassen, 2009).

A short guide on weed control measures that can be applied in amaranth is available on the project website.

1.7.4 Impact of the project

The project has provided significant new knowledge about the importance of weeds in amaranth and their control. Information is now available on the competitveness of amaranth against weeds and the options available to farmers to reduce the adverse impact of weeds on amaranth yields. More specifically, information is now available on the selectivity of herbicides in amaranth, and this information can be used when applications are submitted for regular or off-label registration of herbicide in amaranth. Weeds will be a major constraint to the profitability of amaranth production, but the project has provided new insight into how the adverse effects of weeds can be eliminated or at least reduced.

1.8 Storage of data and multivariate statistical analysis

1.8.1 Objective

The general aims were to assist in the statistical evaluation of the results of the field trials-related results and to provide a publicly available database containing key experimental results. These overall aims were translated into four specific tasks: **1)** assist in designing compatible field trials; **2)** perform a joint analysis of the field trials in collaboration with the groups that generated the data; **3)** perform a multivariate statistics of results of other aspects investigated in the project and **4)** establish a publicly available database on the website of the project.

1.8.2 Methodology

Assistance in designing compatible field trials and implementing a database gathering and storing the data

The field trials were organised with a common experimental design (block design with randomised disposition of parcels with different genotypes in each block) in such a way that the results obtained at five locations (Argentina, Mexico, Spain and two localities of the Czech Republic: Prague and Olomouc) and in two consecutive years were compatible and could be jointly analysed. The experimental data were collected and stored in a common database (available to other participants of the project).

Joint analysis of the field trials (in collaboration with the groups that generated the data and multivariate statistical analyses)

The statistical analyses of the key results directly obtained from the field trials were performed using a common database. Here several production-related parameters (e.g. grain production, harvest index, inflorescence length, etc.) were compared among years and places for each of the 18 genotypes studied separately, by using suitable, generalised linear models (e.g. a gamma model was used for the grain production). Additionally, statistical multivariate techniques were used to jointly characterise the interrelationships among those production-related traits, by using multivariate generalised linear mixed models and covariance selection models. As a sub-product of the project, we could calculate the rough estimates of the heritability of the observed traits for which observations of several individual plants were made.

Establishment of a publicly available database on the website of the project

The key results of the field trials were condensed and placed on a provisory, protected website. These results will be made publically available via the Internet as soon as the analyses are formally published.

1.8.3 End results

The results of the field trials demonstrate that amaranth can be cultivated in a wide variety of agronomic scenarios. Indeed, it was possible to produce amaranth under very different soil and climate conditions, as demonstrated by the field trials. This is not surprising since amaranth is a genus of invasive plants. However, the amaranth culture was shown to be technically challenging and some of the genotypes studied presented production instability. This instability can be seen from the fact that the field trials presented many missed parcels and from the presence of significant statistical interaction (or effect modification) between year and place

as exemplified below. As an example consider the analysis of the grain production (kg/ha) displayed in Table 1.8.1. A joint model for the grain production considering year, place and genotype presented a interaction statistically significant (p-value = 4.4 10^{-07}), indicating that the different genotypes behaved differently at different places and years (even the relative position of the genotypes changed significantly from place and years). Moreover, when analysing the grain production of the genotypes separately, we found that the effect of year and place were not additive (with the exception of *A. cruentus* cv. Don Guiem, var Morelos, *A. hypochondriacus* var. Nutrisol and Rojita), i.e. different responses to locality were observed in the two different years (Table 1.8.1). Similar behaviour was observed for other production parameters (see for example results for harvest index displayed in Table 1.8.2).

Using individual plant measurement to some of the agronomic characteristics allowed us to roughly evaluate the heritability of some traits. These estimates are rough, since the experiment was not designed for this purpose; however, they allow us to conclude, for example, that there is enough genetic plasticity in traits of interest such as plant height at harvest and inflorescence length (heritabilities varying from 5 to 23%). This allow us to preliminarily conclude that it is possible improve amaranth cultivars using classic plant breeding techniques (Labouriau *et al.*, 2010a; 2010b; 2010c; 2010d).

1.8.4 Impact of the project

The field trials (see chapter 1.4) are expected to have substantial impact in the research on cultivation of amaranth, since there are no other studies comparing the agronomic performance of such a wide number of genotypes exposed to such a ample range of field conditions. The statistical evaluation of our results demonstrate that the amaranth culture is flexible enough for being feasible in many different environmental and climatic conditions, but also that more research in the area will be required. We demonstrated that genetic improvement of amaranth varieties is possible and that some important traits for commercial explotation of amaranth (such as plant height and inflorescence length) can respond well to breeding programmes. All in all, it is our expectation that the result of the field trials will serve as a basis for further research in the area.

Table 1.8.1. Grain yield in kg/ha and s.e. (in parentheses). Pairwise comparisons (under a Gamma one-way classification model) via likelihood ratio tests between years for the same place and genotype shown as upper indices following the convention for the level of significance: ** = 1%; * = 5%; ^{n} = not significant and $^{-}$ = not available. Likelihood ratio-based comparisons (under a Gamma two-ways classification model) between places at the same year for each genotype displayed with letters as sub-indices. All the tests are adjusted for multiple comparisons via FDR correction.

2007					
Genotype	**Argentina**	**Mexico**	**Olomouc**	**Prague**	**Spain**
A. cruentus Mexicano	353.5 (154.3)$^{**}_{a}$	310.5 (134.8)$^{n}_{a}$	1595.9 (480.3)$^{n}_{b}$	2007.3 (1178.8)$^{n}_{b}$	4633.3 (390.3)$^{n}_{c}$
A. cruentus R 127	350.0 (33.6)$^{**}_{a}$	506.0 (183.7)$^{n}_{a}$	1162.8 (207.9)$^{n}_{b}$	2146.1 (340.2)$^{n}_{c}$	5257.5 (607.0)$^{n}_{d}$
A. pumilus RAFIN K 340	1102.0 (530.0)$^{n}_{a}$	844.2 (353.3)$^{**}_{a}$	1381.7 (591.4)$^{n}_{a}$	3738.1 (2524.5)$^{n}_{a}$	3460.0 (362.4)$^{n}_{a}$
A. cruentus L. var. Amont	1451.5 (170.0)$^{*}_{a}$	574.2 (91.0)$^{n}_{b}$	2010.1 (707.0)$^{n}_{a}$	3671.9 (847.3)$^{n}_{c}$	5250.0 (1072.3)$^{**}_{c}$
A. caudatus L. CAC 48A	1417.2 (212.8)$^{**}_{a}$	508.0 (31.1)$^{n}_{b}$	1552.5 (174.0)$^{n}_{a}$	4971.3 (227.4)$^{n}_{c}$	5693.8 (960.6)$^{**}_{c}$
A. cruentus cv. Don Guiem	1059.6 (170.0)$^{n}_{a}$	493.2 (105.0)$^{n}_{b}$	2168.4 (625.9)$^{n}_{c}$	4314.2 (2095.0)$^{n}_{c}$	3626.7 (638.3)$^{n}_{c}$
A. hypochondriacus var. Revancha	1060.6 (105.7))$^{-}_{a}$	331.5 (258.4)$^{n}_{a}$	- (-) -$_{-}$	- (-) -$_{-}$	3674.7 (774.1) -$_{-}$
A. hypochondriacus var. Nutrisol	1516.2 (147.1)$^{n}_{-}$	408.2 (22.9)$^{n}_{-}$	- (-) -$_{-}$	- (-) -$_{-}$	- (-) -$_{-}$
A. cruentus var. Tarasca	1870.7 (260.0)$^{n}_{a}$	429.5 (125.9)$^{n}_{b}$	1346.1 (218.5)$^{n}_{a}$	4554.2 (779.0)$^{*}_{c}$	4160.6 (851.4)$^{n}_{c}$
A. cruentus var. Morelos	1273.7 (498.5)$^{n}_{-}$	601.5 (91.0)$^{n}_{-}$	- (-) -$_{-}$	- (-) -$_{-}$	- (-) -$_{-}$
A. hybridus K 593	1274.7 (1005)$^{n}_{a}$	837.0 (197.0)$^{n}_{a}$	1402.8 (199.2)$^{n}_{a}$	3974.4 (1209.8)$^{n}_{b}$	4191.9 (381.0)$^{n}_{b}$
A. hypochondriacus 280 FK-FH1	1561.6 (160.0)$^{n}_{a}$	238.2 (56.0)$^{**}_{b}$	1478.3 (499.4)$^{n}_{a}$	2313.8 (519.3)$^{n}_{a}$	2655.6 (708.8)$^{n}_{a}$
A. cruentus cv. Don Leon	1607.6 (396.4)$^{n}_{a}$	465.5 (42.3)$^{n}_{b}$	1534.7 (75.1)$^{n}_{a}$	3069.0 (543.1)$^{n}_{a}$	5045.6 (332.0)$^{**}_{a}$
A. cruentus cv. Candil	1560.6 (381.4)$^{n}_{a}$	318.8 (65.7)$^{n}_{b}$	1553.3 (81.2)$^{n}_{a}$	3014.6 (2101.0)$^{n}_{ac}$	5593.9 (1421.0)$^{**}_{c}$
A. hypochondriacus San Antonio	1282.8 (242.8)$^{-}_{-}$	613.7 (155.0)$^{n}_{-}$	- (-) -$_{-}$	- (-) -$_{-}$	- (-) -$_{-}$
A. hypochondriacus Rojita/Rosita	1434.3 (162.8)$^{n}_{-}$	481.2 (73.4)$^{n}_{-}$	- (-) -$_{-}$	- (-) -$_{-}$	- (-) -$_{-}$
A. mantegazzianus cv. Don Juan	1008.1 (157.1)$^{n}_{-}$	757.8 (222.7)$^{n}_{-}$	- (-) -$_{-}$	- (-) -$_{-}$	- (-) -$_{-}$

2008					
Genotype	**Argentina**	**Mexico**	**Olomouc**	**Prague**	**Spain**
A. cruentus Mexicano	2921.8 (434.1) $_{e}$	280.5 (112.3) $_{f}$	1596.3 (171.0) $_{e}$	3789.1 (360.4) $_{e}$	3239.0 (209.3) $_{e}$
A. cruentus R 127	2512.5 (906.9) $_{e}$	539.8 (470.8) $_{f}$	2368.0 (388.0) $_{e}$	2839.8 (1172.0) $_{e}$	2342.3 (798.9) $_{e}$
A. pumilus RAFIN K 340	2970.5 (320.8) $_{e}$	272.0 (149.5) $_{f}$	1761.7 (217.5) $_{e}$	2608.9 (843.4) $_{e}$	2316.2 (734.8) $_{e}$
A. cruentus L. var. Amont	2750.2 (411.6) $_{e}$	736.5 (202.0) $_{f}$	1895.6 (370.4) $_{e}$	2631.4 (675.2) $_{e}$	2342.8 (443.9) $_{e}$
A. caudatus L. CAC 48A	3245.5 (576.2) $_{e}$	450.5 (169.7) $_{f}$	2115.7 (784.9) $_{e}$	3666.0 (873.3) $_{e}$	3059.0 (693.3) $_{e}$
A. cruentus cv. Don Guiem	2578.2 (385.3) $_{e}$	548.5 (524.7) $_{f}$	2217.1 (519.3) $_{e}$	2664.8 (929.3) $_{e}$	2166.8 (844.5) $_{e}$
A. hypochondriacus var. Revancha	- (-) -	850.0 (326.1) -	- (-) -	- (-) -	- (-) -
A. hypochondriacus var. Nutrisol	1338.0 (393.4) $_{e}$	501.5 (169.7) $_{f}$	- (-) -	- (-) -	1197.5 (267.3) -
A. cruentus var. Tarasca	3609.1 (633.4) $_{e}$	391.0 (65.1) $_{f}$	2228.6 (347.6) $_{e}$	2336.8 (1304.2) $_{e}$	2824.2 (933.2) $_{e}$
A. cruentus var. Morelos	1600.5 (451.4) $_{e}$	705.5 (204.7) $_{f}$	- (-) -	- (-) -	- (-) -
A. hybridus K 593	3514.2 (426.2) $_{e}$	433.5 (399.8) $_{f}$	1786.3 (473.1) $_{e}$	2807.8 (1058.0) $_{e}$	2244.0 (560.7) $_{e}$
A. hypochondriacus 280 FK-FH1	1886.8 (327.6) $_{e}$	102.0 (48.1) $_{f}$	1977.9 (304.4) $_{e}$	1739.2 (178.7) $_{e}$	1623.8 (695.2) $_{e}$
A. cruentus cv. Don Leon	2203.2 (445.3) $_{e}$	493.0 (90.0) $_{f}$	1284.3 (295.0) $_{e}$	2944.0 (131.0) $_{e}$	2329.2 (563.5) $_{e}$
A. cruentus cv. Candil	3203.0 (734.7) $_{e}$	306.0 (212.3) $_{f}$	2145.0 (198.9) $_{e}$	3166.2 (594.1) $_{e}$	2204.5 (403.5) $_{e}$
A. hypochondriacus San Antonio	- (-) -	610.1 (272.7) -	- (-) -	- (-) -	- (-) -
A. hypochondriacus Rojita/Rosita	2210.5 (473.1) -	532.4 (270.7) -	- (-) -	- (-) -	- (-) -
A. mantegazzianus cv. Don Juan	1995.5 (166.9) -	552.5 (169.7) -	- (-) -	- (-) -	2337.8 (778.1) -
A. hypochondriacus cv. Artasa 122	4147.0 (372.3) $_{e}$	442.0 (394.6) $_{f}$	2024.6 (537.9) $_{e}$	3545.0 (1580.9) $_{e}$	2485.5 (959.3) $_{e}$

Table 1.8.2. Mean harvest index (s.e.) for the 18 studied genotypes in two years and four locations.

2007				
Genotype	**Argentina**	**Mexico**	**Olomouc**	**Prague**
A. cruentus Mexicano	0.056(0.001)	0.200(0.063)	0.407(0.067)	0.113(0.042)
A. cruentus R 127	0.088(0.016)	0.196(0.077)	0.467(0.023)	0.147(0.015)
A. pumilus RAFIN K 340	0.068(0.022)	-	0.373(0.055)	0.143(0.006)
A. cruentus L. var. Amont	0.092(0.012)	0.415(0.173)	0.380(0.235)	0.173(0.081)
A. caudatus L. CAC 48A	0.060(0.010)	0.268(0.064)	0.503(0.060)	0.147(0.032)
A. cruentus cv. Don Guiem	0.047(0.005)	0.354(0.155)	0.497(0.051)	0.127(0.049)
A. hypochondriacus var. Revancha	0.095(0.006)	0.308(0.166)	-	-
A. hypochondriacus var. Nutrisol	0.092(0.012)	0.190(0.034)	-	-
A. cruentus var. Tarasca	0.081(0.014)	0.235(0.070)	0.427(0.049)	0.133(0.023)
A. cruentus var. Morelos	0.084(0.010)	0.224(0.005)	-	-
A. hybridus K 593	0.046(0.006)	-	0.400(0.066)	0.157(0.059)
A. hypochondriacus 280 FK-FH1	0.095(0.006)	-	0.237(0.127)	0.233(0.025)
A. cruentus cv. Don Leon	0.077(0.008)	0.294(0.077)	0.277(0.070)	0.137(0.032)
A. cruentus cv. Candil	0.069(0.003)	0.160(0.047)	0.453(0.023)	0.173(0.076)
A. hypochondriacus San Antonio	-	0.254(0.088)	-	-
A. hypochondriacus Rojita/Rosita	0.048(0.001)	0.167(0.037)	-	-
A. mantegazzianus cv. Don Juan	0.074(0.004)	-	-	-
A. hypochondriacus cv. Artasa 122	0.044(0.001)	-	0.433(0.035)	0.083(0.064)
2008				
Genotype	**Argentina**	**Mexico**	**Olomouc**	**Prague**
A. cruentus Mexicano	0.078(0.008)	0.063(0.025)	0.127(0.021)	0.173(0.031)
A. cruentus R 127	0.054(0.004)	0.100(0.040)	0.117(0.055)	0.240(0.030)
A. pumilus RAFIN K 340	0.051(0.006)	0.126(0.070)	0.107(0.015)	0.100(0.050)
A. cruentus L. var. Amont	0.039(0.003)	0.173(0.070)	0.147(0.074)	0.250(0.069)
A. caudatus L. CAC 48A	0.056(0.004)	0.104(0.027)	0.107(0.021)	0.210(-)
A. cruentus cv. Don Guiem	0.059(0.003)	0.127(0.074)	0.157(0.092)	0.200(0.026)
A. hypochondriacus var. Revancha	-	0.230(0.057)	-	-
A. hypochondriacus var. Nutrisol	0.062(0.006)	0.106(0.041)	-	-
A. cruentus var. Tarasca	0.072(0.010)	0.142(0.109)	0.123(0.045)	0.167(0.104)
A. cruentus var. Morelos	0.077(0.012)	0.124(0.007)	-	-
A. hybridus K 593	0.067(0.009)	0.272(0.268)	0.093(0.046)	0.167(0.021)
A. hypochondriacus 280 FK-FH1	0.049(0.003)	0.067(0.016)	0.103(0.112)	0.150(0.062)
A. cruentus cv. Don Leon	0.054(0.004)	0.138(0.049)	0.140(0.053)	0.233(0.032)
A. cruentus cv. Candil	0.065(0.008)	0.091(0.077)	0.157(0.064)	0.130(0.056)
A. hypochondriacus San Antonio	-	0.106(0.038)	-	-
A. hypochondriacus Rojita/Rosita	0.057(0.005)	0.102(0.041)	-	-
A. mantegazzianus cv. Don Juan	0.048(0.007)	0.202(0.020)	-	-
A. hypochondriacus cv. Artasa 122	0.053(0.004)	0.159(0.061)	0.133(0.031)	0.327(0.150)

1.9 Amaranth cultivation in women's agricultural cooperatives

1.9.1 Objective

The objective of this contribution was to empower Nicaraguan single provider women in rural areas to achieve improved food security through the introduction of selected amaranth species and to provide an alternative crop in former cotton growing areas of Nicaragua and thus reduce the environmental impact of heavy uses of pesticides. These objectives were reached through the following tasks: **1)** search for indigenous amaranth species in Nicaragua; **2)** introduce amaranth as a rotation harvest in former cotton cultivation areas in Nicaragua in a women's agricultural cooperative; **3)** standardise some methodologies to extract and quantify organochlorine pesticides and toxaphene congeners in grains, leaves, stem and root of Amaranth plants and in the soil during the growth and after the Amaranth harvest; **4)** disseminate results in Nicaragua and the neighbouring countries and **5)** evaluate the food quality of Nicaragua-adapted recipes with amaranth.

1.9.2 Methodology

Introduction of amaranth cultivation in women's cooperatives

Two rural cooperatives of women from communities La Tejana and La Bolsa were involved, the majority of them single mothers with an average of six children. In general the women earn their living either as maids, cooks and nannies in the urban area or as manual labourers in the agricultural field. Every group had a piece of land where two small studies were conducted. The objective was to demonstrate the agronomic plant health, the costs of production and the importance of amaranth as far as food security and nutrition are concerned. The menu was offered to provide examples of how to integrate amaranth into the traditional Nicaraguan kitchen. Included in all of these activities were local and national government organisations, university level agricultural programmes and nongovernmental organisations related to food security, nutrition and agriculture.

1.9.3 End results

Three species of indigenous amaranth were collected in different locations in the country, two with thorns and one without. The taxonomic classification was done by MSc Alfredo Grijalva, a botanist with 35 years of experience. Two species were collected in two different communities of the municipality of Chinandega, the first called Pellizco Occidental, which is 35 kilometres west of the city of Chinandega on the skirts of the San Cristobal Volcano. The second community is called Cinco Cruces (Five Crosses) and is only 15 kilometres away from the city of Chinandega.

Species No 1. *Amaranthus viridis*
L. Found in the Pellizco Occidental Community, Municipality of Chinandega, Scientific Name: *Amaranthus viridis L.* **Common name:** "Bledo" without thorns. **Location:** community Western Pellisco. **Geographical location:** 12º 40'29″N, 87º00'50″W.

Species No 2. *Amaranthus cruentus*
L. Found in the Cinco Cruces Community, Municipality of Chinandega, Scientific Name: *Amaranthus cruentus L.* **Common name:** "Bledo" without thorns. **Location:** community:

Cinco Cruces. **Geographical location:** 12º 34'49"N, 86º59'56"W.

Species No 3. *Amaranthus cruentus*
L. Found in the Los Chilamates Community, Municipality of Esteli, Scientific Name: *Amaranthus cruentus* L. **Geographical location:** 13º 08'44"N, 86º22'11"W.

Species No 4. *Amaranthus spinosus* L. Found across Nicaragua
Scientific name: *Amaranthus spinosus* L. **Popular name:** Spiny Amaranth, Prickly Amaranth, Thorny Amaranth. **Location:** Across Nicaragua. **Geographical Location:** 11º to 13º N, 83º to 86ºW.

There are 42 women, all mothers and manual labourers, who work together on the Amaranth project (22 in the La Bolsa community and 20 in the La Tejana community). Popular name: Spiny Amaranth, Prickly Amaranth, Thorny Amaranth. Location: Across Nicaragua. Geographical Location: 11º to 13º N, 83º to 86º W. The plots were first established on 2700 square metres of land on 24-29 May 2007. The variety used was A. cruentus, Mexicano. The results from this season were not very good due to the dry weather; September and October are known for heavy precipitation. After this experience, it was determined that planting in the middle of August would yield better results.

The experimental plots were established in La Tejana (May 2008) with 12 genotypes brought from Argentina in an arrangement of "Bloques completo al Azar" (BCA) with four repetitions. The results of the investigation showed that the species of amaranth that adapted best to Nicaragua was the *A. cruentus*, outperforming the *A. cruentus* Mexicano with 1177.40 kg/ha. The others species including *A. hypochondriacus*, *hybridus* and the tricolour yielded than 60-70% less than the *A. cruentus* variety. For any cultivation of this amaranth, first time or large commercial scale, it is not recommendable to plant in September or October due to the heavy rains and occasional flooding.

The establishment of the trial on the conditions of irrigation (December 2007-April 2008) served to obtain information on the work that is carried out in conjunction with the UNAN (Phd. Marta Lacayo) on the absorption of pesticides into the soil.

In the cultivation with irrigation (December 2007-April 2008) three species with the best yield in the previous cycle were evaluated and added to the *A. montegazzianus* species, also brought from Argentina. The trial "Bloques complete al Azar" with 4 repetitions. The only condition was that in this trial the quantity of water used was 700 mm in total in the whole cycle, divided into the different phases of the development of the crop. This was with the objective to test the adaptability of the amaranth species with very little water. In Nicaragua there are micro-zones where it only rains between 900 and 1000 mm, and these regions have serious problems with bad nutrition and food security. Under these conditions of dehydration and stress the species *A. cruentus* had the best results: the *A. cruentus* Mexicano yielded 790.60 kg/ha. Next came *A. cruentus* Don Leon with 712.00 kg/ha. In last place, the species *A. montegazianus* yielded 291.40 kg/ha. This species had problems between December and April, the period with the most direct sunlight, and the tallest plant only reached 25 cm (Espinosa-Pérez *et al.*, 2010).

In the study of the absorption of pesticides in the soil around the amaranth the following results were obtained: all the samples were extracted with hexane and methylene chloride for 8 hours. The samples were evaporated and a sulphur destruction was carried out, then the samples were cleaned up with three kinds of organic solvents. The samples were evaporated and then they were injected in MS/GC to quantify organochlorine pesticides and toxaphene congeners. The total samples of amaranth plants analysed were 80, and 48 samples to evaluate the quality control were done. The species investigated to measure the pesticides were: *A. cruentus* Mexicano, *A. caudatus* CAC48 Perú and *A. cruentus* Don León. The plant parts studied were roots, stem, leaves, grains. Soil samples were studied also. The pesticides analysed were: toxaphene and Parlar: 11, 12, 15, 31, 32, 39, 40, 42, 44, 50, 59 and 63. α-HCH, β-HCH, γ-HCH, aldrin, dieldrin heptachloro, heptachloro-epoxide endosulphane, β-endosulphane, pp-DDT, pp-DDD, pp-DDE The pesticides found in the three species were: Parlar-40, H-epoxide, α-HCH, β-endosulfan, Parlar-50, dieldrin, Parlar-39, Parlar-15, aldrin and γ HCH and β-HCH Lacayo-Romero *et al.*, 2010). Phytoremediation and degradation of other organic compounds and heavy metals with some amaranth species have been achieved by several procedures. However, studies aiming at toxaphene degradation employing *A. cruentus* Mexicano, *A. caudatus* CAC48 Perú and *A. cruentus* Don León have been scant. To our knowledge, it is the only example reported to degrade the toxaphene congeners with these amaranth species used.

In developing countries like Nicaragua these results provide a cost-effective method for the removal of toxaphene due to the low cost and the possibility to carry out the biodegradation process in a single step process. However, further studies must be done in order to clarify the real mechanism involved in the degradation of these organic pollutants.

The diseases that were monitored during the different phases of crop development were:
Development phase: order Lepidoptera and order Coleoptera. In this phase, the affected area which included up to 30% of the crop did not significantly decrease the yield.
Flowering phase: order Hemiptera, Acaros (red spiders) of the order Coleoptera and the corn worm (*Spodoptera frujiperda*). In this period, the affected area did exceed 30%, threatening the yield.

In the meetings and training seminars, producers participated in the elaboration of crop management techniques and plans. Production costs of amaranth were also discussed. It was determined that the cost of establishing 1 ha is US$ 258.00. Additionally, it was calculated that if one produces 11 hkg/ha, there is a margin of net gain of US$ 732.00 before subtracting the pre-cultivation and transportation costs.

1.9.4 Impact of the project

The dissemination of the results of the study is on-going. Governmental and non-governmental organisations that work with food security and nutrition are involved, as well as national scientific committees who are making their presence known throughout Nicaragua. With these organisations, no formal plan or contract has been established due to lack of funding from all sides. The best way to spread the information is to go to rural areas and work with their local organisations to promote amaranth as a crop, highlighting the agronomic potential and nutritional content compared to the other grains currently cultivated (corn, beans, sorghum

and wheat). It is also important to highlight the different ways that amaranth can be used in daily food preparation; there are at least 18 different ways to incorporate it. To carry out these training sessions, materials such as seeds and informational pamphlets would be crucial. Verbal contact is maintained with: PROMIPAC (integrated disease management programme in Latin America), FUNICA (foundation for the development of agriculture and livestock as well as forest technology in Nicaragua), CLUSA (association of leagues of cooperatives), SAVE THE CHILDREN, EL INTA (Nicaraguan institution of agriculture and livestock-technology) and FODEL (federation of NGOs for the local development in Nicaragua: a group of private producers interested in planting for cattle feeding purposes).

1.10 References (scientific publications from the AMARANTH:FUTURE-FOOD project)

Aguilar-Hernández HS, León-Galván MF, Guevara-González RG, Barrera-Pacheco A, Espitia-Rangel E & Barba de la Rosa AP. 2010. Characterization of differential transcripts in *Amaranthus hypochondriacus* L involved in salt stress response. (In prep., 2010).

Barba de la Rosa AP, Fomsgaard IS, Laursen B, Mortensen AG, Olvera-Martínez JL, Silva-Sánchez C, Mendoza-Herrera A, González-Castañeda J & De León-Rodríguez A. 2009. Amaranth (*Amaranthus hypochondriacus*) as an alternative crop for sustainable food production: Phenolic acids and flavonoids with potential impact on its nutraceutical quality. *Journal of Cereal Science* 2009, *49*, 117-121.

Barba de la Rosa AP, Barba-Montoya A, Martínez-Cuevas P, Hernández-Ledesma B, De León-Rodríguez A & González C. 2010. Tryptic amaranth glutelin digests induce endotelial nitric oxide production through inhibition of ACE: antihypertensive role of amaranth peptides. *Nitric Oxide* 2010, *23*, 106-111.

Barrio DA & Añón MC. 2010. Potential antitumor properties of a protein isolate obtained from the seeds of Amaranthus mantegazzianus. *European Journal of Nutrition* 2010, *49*, 73-82.

Casarrubias-Castillo K, Estrada-Hernández MG, Martínez-Gallardo NA & Délano Frier JP. 2009. Bacteriosis resistance in genotypes of amaranth (*Amaranthus spp.*) of agronomic interest. XIII National Congress of Biochemistry and Plant Molecular Biology & 6th Symposium Mexico-USA. Guanajuato, Gto., Mexico. November, 2009.

Condés MC, Scilingo AA & Añón MC. 2009. Characterization of amaranth proteins modified by trypsin proteolysis. Structural and functional changes. *LWT* 2009, *42*, 963-970.

Cruz-Ortega LB, León-Galván MF, Huerta-Ocampo JA & Barba de la Rosa AP. 2010. Cloning and molecular characterization of Cu,Zn Superoxide dismutase from amaranth (*Amaranthus hypochondriacus* L.) expressed under abiotic stress. (In prep., 2010).

de Troiani RM, Reinaudi N & Troiani H. 2008. Efecto del control mecánico de malezas en los caracteres agronómicos de tres genotipos de amaranto. *Revista de Desarrollo Rural y Cooperativismo Agrario* 2007-2008, *11*, 19-30.

Délano Frier JP. 2008. Amaranto: alimento del futuro. XIII Simposium Internacional de Ingeniería en Industrias Alimentarias: Fusión. Instituto tecnológico y de Estudios Superiores de Monterrey, Campus Querétaro. October 2008.

Délano Frier JP. 2009a. Amaranth: cultivation, pests, pathogens and tolerance to stress conditions. Jornadas 09, Amaranto. La Plata, Argentina. October, 2009.

Délano Frier JP. 2009b. Hallazgos en el transcriptoma del amaranto. Simposio Científico, Amaranto 2009. Querétaro, Qro., Mexico. November, 2009.

Espinosa-Pérez JF, Fonseca-Trujillos AR, Lacayo-Romero ML, Arguello-Montiel LM, Esquivel-Quezada JL, Hernández-Norori MA, Jaquín-Pascua MC & Uriarte-Ortiz RJ. 2010. Agronomic experiences in different Amaranth species cultivated in dry soils from Nicaragua. (In prep., 2010).

Fritz M, Vecchi B, Rinaldi G & Añon MC. 2010. Amaranth seed protein hydrolysates have in vivo and in vitro antihypertensive activity. (Submitted).

Huerta-Ocampo JA, Briones-Cerecero EP, Mendoza-Hernández G, De León-Rodríguez A & Barba de la Rosa AP. 2009. Proteomic analysis of amaranth (*Amaranthus hypochondriacus* L.) leaves under drought stress. *International Journal of Plant Science* 2009, *170*, 990-998.

Huerta-Ocampo JA, León-Galván MF, Cruz-Ortega LB, Barrera-Pacheco A, De León-RodríguezA, Mendoza-Hernández G & Barba de la Rosa AP. 2010. Water stress induces up-regulation of DOF1 and MIF1 transcription factors and down regulation of proteins involved in secondary metabolism in amaranth roots (*Amaranthus hypochondriacus* L.). *Plant Biology*. doi: 10.1111/j.1438-8677.2010.00391.x..

Janovská D, Stavělíková H & Dušek K. 2008. Field evaluation of amaranth in the Czech Republic. In: Libiakova and Gajdošova (ed.) Amaranth – Plant for the future. 5th International Symposium of the European Amaranth Association. Nitra, 9-14 November, 2008, Slovak Republic, 70-73 pp.

Kudsk P & Mathiassen SK. 2010. Weed control in amaranth, In prep.

Kudsk P, Mathiassen SK & Taberner A. Survival of seeds of cultivated amaranth species. (In prep.).

Labouriau RS, Délano Frier JP, Janovska D, Noellemeyer E, Reinaldi N, Taberner A, & Troiani RM. 2010a. Field evaluation of 18 genotypes of grain Amaranth in five different environments. (In prep.).

Labouriau RS, Délano Frier JP, Dusek K, Janovska D, Noellemeyer E, Taberner A, Stavelikova H & Troiani RM. 2010b.Comparison of 18 Amaranth genotypes in 2 years and 5 places (4 countries). (In prep.).

Labouriau RS, Délano Frier JP, Dusek K, Janovska D, Noellemeyer E, Taberner A, Stavelikova H & Troiani RM. 2010c. Growth model for predicting Amaranth production. (In prep.).

Labouriau RS, Délano Frier JP, Dusek K, Janovska D, Noellemeyer E, Taberner A, Stavelikova H & Troiani RM. 2010d. On the heritability of key agronomic traits for 18 genotypes. (In prep.).

Lacayo-Romero ML, Arguello-Montiel LM, Esquivel-Quezada J, Hernández-Norori MA, Jaquín-Pascua MC, Uriarte-Ortiz RJ, Fonseca-Trujillos AR & Espinosa-Pérez J. 2010. Comparation of three Amaranth species capable to degrade organochlorine pesticides in contaminated soils from Nicaragua. (In prep., 2010).

Mathiassen SK, Kudsk P & Fomsgaard IS. 2008. *Allelopathy in cultivated amaranth varieties*, Abstract and poster, International Allelopathy Congress, Saratoga Springs, New York, USA. 20-25 September.

Mathiassen SK & Kudsk P. 2009. *Herbicide selectivity in amaranth*, Abstract and poster, Jornadas 09 Amaranto, La Plata, Argentina. 22-23 October.

Mathiassen SK, Kudsk P & Fomsgaard IS. 2009. *Allelopathy in cultivated amaranth varieties*, Abstract and poster, Workshop Jornadas 09 Amaranto, La Plata, Argentina. 22-23 October.

Mathiassen SK & Kudsk P. 2010. Competitive ability of different Amaranth species/varieties (In prep).

Navarro-Meléndez A L, Martínez-Gallardo N A, Valdés-Rodríguez S & Délano Frier JP. 2008. Proteomic analysis of amaranth leaves (*Amaranthus spp.*) subjected to insect herbivory or treated with methyl jasmonate, an elicitor of insect resistance. The 2008 Joint Annual Meeting of the American Society of Plant Biologists and the Sociedad Mexicana de Bioquímica. Mérida, Yuc. June/July 2008.

Navarro-Meléndez A, Martínez-Gallardo N, Valdés Rodríguez SE & Délano-Frier JP. 2009. Proteomic analysis of amaranth leaves (Amaranthus hypochondriacus) subjected to insect herbivory or treated with methyl jasmonate, an elicitor of resistance responses. XIII National Congress of Biochemistry and Plant Molecular Biology & 6th Symposium Mexico-USA. Guanajuato, Gto., Mexico. November, 2009.

Ochoa-Sánchez JC, Parra-Cota FI, Aviña-Padilla K, Délano-Frier J & Martínez-Soriano JP. 2009. *Amaranthus spp.*: a new host of "*Candidatus* Phytoplasma aurantifolia". *Phytoparasitica* 2009, 37, 381-384.

Parra-Cota FI, Martínez-Soriano JP & Délano-Frier JP. 2009. Molecular analysis of the causal agent of the Phytoplasma disease in *Amaranthus spp.* XIII National Congress of Biochemistry and Plant Molecular Biology & 6th Symposium Mexico-USA. Guanajuato, Gto., Mexico. November, 2009.

Pedersen HA, Steffensen SK, Christophersen C, Mortensen AG, Jørgensen LN, Niveyro S, de Troiani RM, Rodríguez-Enríquez RJ, Barba de la Rosa AP & Fomsgaard IS. 2010 Synthesis and quantitation of six phenolic amides in *Amaranthus spp.* (Submitted, 2010).

Repollo R, de Troiani RM, Nollemeyer E, Sánchez TM & Reinaudi NB. 2010. Respuesta del amaranto a dos distancias de siembra y a densidades de población. (In prep., 2010).

Sánchez T, de Troiani RM & de Ferramola LA. 2007. Aplicación de nitratos y cloruros en Amaranthus tricolor L. Efecto sobre la producción de materia verde, materia seca, contenido de nitratos y cloruros. *Rev. FCACuyo.* Tomo XXXIX, *Nº 2*, 35-42.

Silva-Sánchez C, Barba de la Rosa AP, León-Galván MF, de Lumen BO, De León-Rodríguez A & González de Mejía E. 2008. Bioactive peptides in amaranth (*Amaranthus hypochondriacus*) seed. *Journal of Agricultural and Food Chemistry* 2008, *56*,1233-1244.

Steffensen SK, Rinnan Å, Mortensen AG, Laursen B, de Troiani RM, Noellemeyer E, Taberner A, Janovska D, Dusek K, Délano-Frier J, Christoffersen C & Fomsgaard IS. 2010. Phenolics in amaranth seeds. (Submitted, 2010a).

Steffensen SK, Rinnan Å, Mortensen AG, Laursen B, Troiani RM, Noellemeyer E, Taberner A, Janovska D, Stavelikova H, Pedersen HA, Christoffersen C & Fomsgaard IS. 2010. Identification and quantification of flavonoids, phenolic acids, cinnamoylamides, trigonellin and betain in vegetable amaranth species. (In prep., 2010b).

Steffensen SK, Mortensen AG, Laursen B, de Troiani RM, Noellemeyer E, Taberner A, Janovska D, Christoffersen C & Fomsgaard IS. 2010. Vegetable amaranth species contain varying concentrations of a rare terpene, a rare phenolic compound and two saponins. (In prep., 2010c).

Steffensen SK, Añon CM, Barrio D, Spirero F, Martinez N & Christophersen C. 2010. Proliferation studies on Amaranth extracts and Standards. (In prep., 2010d).

Taberner A, Zamora N, Llenes JM & Roque A. 2009. Use of the flex-tine harrow for grain production in an Amaranth crop. Proceedings 8th EWRS Workshop on Physical and Cultural Weed Control Zaragoza, Spain, 9-11 March 2009, p. 80.

Tironi VA & Añón MC. 2010. Amaranth proteins as a source of antioxidant peptides: Effect of proteolysis. *Food Research International* 2010, *43*, 315-322.

Vargas-Ortiz E, Martínez-Gallardo NA & Délano-Frier JP. 2008. Analysis of carbohydrate and nitrogen re-allocation as an herbivory tolerance mechanism in grain amaranth. The 2008 Joint Annual Meeting of the American Society of Plant Biologists and the Sociedad Mexicana de Bioquímica. Mérida, Yuc. June/July 2008.

Vargas-Ortiz E, Martínez-Gallardo NA & Délan-Frier JP. 2009. Analysis of carbohydrate and nitrogen allocation in response to herbivore attack in grain amaranth (*Amaranthus cruentus*). XIII National Congress of Biochemistry and Plant Molecular Biology & 6th Symposium Mexico-USA. Guanajuato, Gto., Mexico. November, 2009.

Vargas-Ortiz E, Martinez-Gallardo NM, Tiessen A & Delano Frier JP. 2010. Partial defoliation by herbivore attack alters carbohydrate allocation to different tissues of grain amaranth (*Amaranthus cruentus*). (Submitted, 2010).

Vecchi B & Añón MC. 2009. Two novel anti hypertensive tetrapeptides from *Amaranthus Hipochondriacus* 11S globulin. *Phytochemistry* 2009, ***70***, 864-870.

Ventureira J, Martínez EN & Añón MC. 2010. Stability of oil: Water emulsions of amaranth proteins. Effect of hydrolysis and pH. *Food Hydrocolloids* 2010. ISSN: 0268005X. DOI: 10.1016/j.foodhyd.2009.12.008. ISSN: 0268005.

Zamora N, Llenes JM, Roque A & Taberner A. 2008. Productividad de 18 poblaciones de Amaranthus, cultivo para producción de alimento exento de gluten, y malas hierbas que le afectan. Procedings XXVII Reunión del Grupo de Trabajo de "Malas Hierbas y Herbicidas" de las C.C.A.A., Vitoria, 2008.

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