

CONTEMPORARY CHALLENGES IN SUNFLOWER BREEDING

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ABSTRACT

The discovery of novel agronomically- and commercially-significant traits has changed the segmentation of the sunflower crop over the last years and has triggered higher complexity in sunflower breeding. Further, the development of the palm oil sector has changed the vegetable oil market. To remain competitive in this market, sunflower breeding needs to accelerate efforts to improve yield potential as well as adaptation to abiotic-/biotic-stress. Molecular genetics and associated technologies play significant roles in the discovery and understanding of novel traits. Together with high-throughput sequencing, molecular technologies will drive breeding to high-efficiency in the current era. Marker Assisted Recurrent Selection (MARS) and Genome Wide Selection (GWS) will allow breeders to better predict the breeding value, as manifest by genetic gain, of crosses and breeding programs. The application of new tools (*e.g.* digital phenotyping, hyperspectral-imaging, drones, and tractor-sensory-platforms) to facilitate complex data acquisition will significantly improve the precision of measurements and increase breeding efficiency. Interspecific-hybridization and induced mutations are, and will continue to be, major development areas for the identification of new traits and crop improvement. Doubled-haploidization (DH) is a methodology currently unavailable to the sunflower breeding process. This is likely to remain the situation for the foreseeable future as the development of DH for sunflower faces substantial technical and biological challenges. In January 2015, the “bronze” version of the first genomic reference sequence for sunflower (based on accession HA412-HO) was published and, by June, made publicly available. This milestone represents a clear advantage for sunflower breeding and is the foundation for increasing our knowledge of the sunflower genome. As our understanding of the sunflower genome improves, we will, over the next few years, transition to the post-genomic era in sunflower.

MARKET SEGMENTATION AND BREEDING GOALS

Oil crops take up around 10% of the total cultivable area worldwide, and other than the main cultivation aim, which is the production of oil, they also play an important part in food production, as well as in the sector of energy for the production of biodiesel. On the list of the most important oil crops, sunflowers hold a high position at number four. Allowing for the exception of breeding modifications geared towards the yield of seed and oil as the primary aim of breeding programs in the private sector (the primary goal of non-profit organisations can vary), the increase in market segmentation has had a great impact on breeding goals in the last few years. This refers, in particular, to the increase in demand for sunflower varieties with higher oil yields, as well as the introduction of herbicide-tolerant hybrids. In comparison to the situation 15 to 20 years ago, when there were 12 basic segments, there is greater complexity with 24 basic segments present in the contemporary market. Complexity is further increased with the sub-segmentation of: herbicide-tolerance (HT) between sulphonylurea (SU) and imidazolinone (IMI) resistances; disease races present

in specific regions (*e.g.* downy mildew- or broomrape-races); or by allowing for the segmentation distinguishing between oil production and confectionary as an end-use. This type of segmentation tends to be more global and is not applicable to every market. The segmentation of certain markets is far simpler than that of others: *e.g.* Argentina and the USA have market segmentation patterns that are much simpler than in Europe. This does not, however, diminish the importance of primary breeding goals which are seed- and oil-yield, as well as resistance to economically important diseases. Another segmentation type exists in India, where seasonal cultivation (spring and winter) informs the primary segmentation, and where an HT segment does not functionally exist. On the other hand, in the Russian and Ukrainian markets (which make up over 50% of the total sunflower cultivation areas), nearly all market segments are observed.

Addressing this type of segmentation requires the participation of all functions in the hybrid breeding process ranging from strategic planners, marketing teams, sales managers, research and development teams, production and even external consulting companies which help the team make sound strategic decisions. The programs must be balanced towards certain market segments and financial capabilities. It should never be forgotten that the selective breeding process takes a long time and that any wrong decision made today can have implications which are made visible only after a few years have passed. Although segmentation is relatively stable, it is still dynamic in its own right, and strategic choices are to be adjusted according to the development of certain segments. Sunflower is, above all, an industrial crop whose segment development depends heavily on industrial demand. The increase in demand for high oil-yield sunflower can drastically affect the priorities and strategies of segment development in relation to high oil-yield varieties, especially after the implementation of labeling regulations in the EU. This is particularly important when developing segments for export-oriented markets such as Ukraine and Argentina.

A highly segmented market, such as that of sunflower, can rapidly change the demands placed on breeders and their programs. Further, this environment changes the attitude of breeders themselves, by shifting the process from distinct programs based on the efforts of an individual to team-based programs. Effective delivery requires/will require:

- increases in breeding efficiency;
- the introduction of new methods and techniques;
- expedition of the selection process using methods such as Marker Assistant Selection (MAS) and MARS;
- efficient intra-/extraorganizational material transfer (including the drafting and validation of material transfer agreements where necessary);
- strict breeder rights enforcement wherever possible;
- the creation of joint programs in collaboration with public and non-profit institutions;

BEYOND THE GENE

One of the greatest challenges breeders face is improving the analysis and understanding of gene expression/regulation and the subsequent impact on individual phenotypes, the phenome, and the metabolome given the context of environment. The development of "-omics" data and capabilities will be crucial in the coming decade and help improve the understanding of the complex processes that take place within the whole organism. Genomics, phenomics, and metabolomics have a particular importance in sunflower research going forward.

Phenomics is a relatively new discipline whose name derives from the word "phenome". Coined in middle of the 20th century (Davis, 1949), phenome refers to the set of all potentially observable phenotypes of a cell, tissue, organ, organism, or species. Phenomics explores a wide array of processes influencing the phenome and individual phenotypes. Phenomics can also be defined as a highly detailed dissection of breed traits and their connection to the gene or genes (Furbank and Tester, 2011). The identification of QTL within the genome of differing plant species is a difficult and demanding task and the factors which can influence QTL detection can vary greatly: the genetic QTL basis causal to the trait in question, environment influence, population size, experimental error, and *etc.* The precision of phenotypic rating and the robustness of QTL mapping are intimately linked: a reasonable and actionable QTL map cannot be generated without accurate phenotype rating data. During the last 20 years or so, progress in precision phenotyping was more limited than that of molecular techniques, thus reducing the utility of QTL, especially in relation to traits such as seed yield. The research into *Phoma macdonaldii*, done by Maleki *et al.* (2014), showed a sizable genetic variability within the F₃ generations. 14 main QTLs were found, localized within seven related groups, with a phenotype variation ranging from 4 to 24 percent. The most important QTL identified in this study related to partial resistance to the three fungus isolates applied in the study. This QTL is a strong candidate for introduction of *Phoma macdonaldii* resistance using MAS. Sunflowers were also subjected to QTL research for other traits such as: water content and chlorophyll (Abdi *et al.*, 2012), seed yield traits (Kiani *et al.*, 2009), photosynthesis and CO₂ concentration (Herve *et al.*, 2001), flowering period (Cadic *et al.*, 2013), and overall phenotype (Wills *et al.*, 2010), *etc.*

A number of newer integrated solutions exist to tackle complex measurements and generate robust phenotypic ratings. Phenomics is based on the use of most of these technologies/methodologies: this paper will point out the significance and application aspects of some of the most critical:

Digital phenotyping is one of the cheapest, and most commonly used methods. With the aim of adequately processing digital imagery data and interpreting the results. There are many variants in application in relation to where and how these technologies are being used, dependent upon the crop culture being investigated. The device and methods developed by Van der Heiden *et al.* (2012), is a sterling example of digital phenotyping done on tall cultures in a greenhouse environment. The experiment was done on the recombined inbred lines of peppers, in the aim of determining heritability of QTLs. By applying this technique, three QTLs were determined for leaf size, and one for the leaf angle. Using statistical analysis, it was confirmed that this method has a high degree of correlation (0.93) with empirical, classical measurement of plant height, and total leaf surface.

Hyperspectral imaging or imaging spectroscopy combines the power of digital imaging and spectroscopy. Hyperspectral remote sensors collect image data simultaneously and it makes it possible to derive a continuous spectrum for each image cell. This technology is highly applicable to an array of scientific areas. It is extensively applied in plant disease analysis, as well as drought resilience research. The method proved itself successful in detecting symptoms of different diseases found in sugar beet, because pathogens cause differing spectral signals dependant on interaction with plant tissues (Mahlein *et al.*, 2012).

Drone application: In the past, drones were available mainly for military purposes, but in recent times they have become less expensive and more popular instruments for research, as well as in farmers' daily lives. There are a variety of designs, such as mini fixed-wing aircraft, and miniature multi-propeller helicopters. What all of them have in common is

their miniaturized stature and integrated GPS and camera systems. They are controlled remotely and operated by a single user. Agricultural producers are able to use them to optimize irrigation, detect diseases, and damage in the fields. The use of drones is widespread in a variety of developing projects including precision plant phenotyping.

Studying the germination process using cameras and computer monitoring. Germination is one of the most important processes in production. At the same time it presents a great challenge in precision phenotyping and understanding the vigor phenomenon. Wagner *et al.* (2011) developed a method which represents a combination of the Jakobsen table (germination system known also as the "Copenhagen table" which provide constant temperature and moisture level for all seed samples) and four calibrated cameras which are able to analyze a sample of up to 400 seeds per camera, or individual seeds. Special software was developed for the process in the aim of controlling the germination parameters, as well as taking photographs. Another piece of software, *ImageJ*, analyzes the photographs and turns them into data which is later analyzed in detail. Although primarily developed for the use in sunflower germination, the system has been found to be useful in other plant species as well.

Multi-sensor tractor platforms. Various groups of authors have, by now, developed multifunctional tractor platforms. Equipped with a wide array of sensors, such as 3-D cameras, laser and hyperspectral measurement sensors, *etc.*, the platforms provide an opportunity for the installation of many other types of sensors, depending on the type of research conducted: for instance, moisture content in plants, lodging, biomass yield and similar (Busemeyer *et al.*, 2013). One of the limiting factors is their use on tall cultures at later developmental stages.

METABOLOMICS

Metabolomics is an area of research which combines strategies to identify and quantify cellular metabolites using sophisticated analytical technologies with the application of statistical methods for information extraction and data interpretation (Roessner and Bacic, 2010). Due to the complexity of analytics involved in their determination, a series of extractions, detections, quantification and metabolite identifications must be performed. As metabolite profiles are directly related to phenotype, metabolomics can be widely applied to determine gene functions and of the interaction of function with environmental factors such as biotic and abiotic stress. Today, metabolomics is increasingly used to analyze the tolerance of plants to: increased salinity; low temperatures; micronutrient deficiency; their toxicity, and *etc.* Cultivated sunflower, along with wild relatives, is well known as a rich source of plant metabolites of varying classes such as terpenoids. Broomrape (*Orobancha cumana* Wallr.) is a parasitic flowering plant known to cause extensive damage to sunflower and is probably the biggest obstacle in sunflower cultivation on globally. Very little is currently known about the specific processes involved in the changes in metabolism resulting from the plant's reaction to this parasite. Using the Ultra-High Performance Liquid Chromatography-High Resolution Mass Spectrometry (UPLC-MS) method, A.-L. Hepp *et al.* (2013) distinguished key differences in the metabolism of specific metabolites present after the plant's exposure to the parasite. The biosynthesis of coumarines, lignans, and alkaloids also varied significantly depending on the plant's defense response. This research, and others alike, will significantly help the understanding of plant metabolism when interacting with broomrape and point to a path which can lead to a better means of controlling the parasite.

Uniseriate linear glandular trichomes (LGT) are a part of the stem, leaves, and flower cluster of the *Heliathus* family. Their biological role and metabolic activity is still under investigation. The results demonstrated by Spring *et al.* (2015) pointed to the fact that LGTs

accumulate a variety of sesquiterpens (ST) and that ST synthesis requires sun exposure. Flavonoids are syntesized in parallel with ST, and are presumed to have a protective role against UV radiation. The authors also determined the existence of four known nevandesin types of flavonoids, none of which were found previously in *H. annuus*.

Metabolic markers can potentially be used in plant breeding (Stenfath *et al.*, 2010).

GENOME SELECTION

In the broad sense, a genome is all the genetic material contained within a single organism. Genome-based selection as a methodology is of a more recent date and has great perspectives in the future. Although MARS (Marker Assistant Recurrent Selection) is still a developing method, genome selection (GS) already has the upper hand. While the marker assistant recurrent selection is based solely on markers that bare significant effects, GS encompasses all known markers in the entire genome. A large number of research papers have shown that the application of MARS is more successful than the regular phenotypic selection methods, and that GS is even more successful than MARS, which is especially true for traits that have a multi-genic origin and are heavily influenced by the enviornment. Information relating to the phenotype and genotype of a reference population enables the prediction of model parameters. Models, in turn, predict phenotypes in the plant population based on genotype results and produce genome breeding value (GEBV). This forms the base for selection of target phenotypes (A.M. Pérez-de-Castro *et al.* 2012).

DEVELOPMENT OF DH TECHNOLOGY

The creation of inbred lines using double-haploids has many benefits not only for shortering the plant cycle but haploids can also be utilized to provide researchers with genetic information not possible with normal diploid individuals. Since haploids possess only a single dose of their respective genomes this signifcantly facilitates the search and selection of favorable genes and the development of superior breeding genotypes. Sadly the technology is not available to sunflower breeders yet. By using this technology, it is possible to create homozygote lines in a single generation, thus the requirement for several generations of selfing. A number of published DH protocols exist in other plant cultures and this technology is today used routinely in barley, oilseed rape, wheat, and, more recently, the application of induced DH is being successfully used in corn. After years of research into anther culture, microspore cultures or radiation induced haploids, it can be concluded that none of the methods listed have shown promising results in sunflower, but there is hope that further research into radiation induced haploidisation will bare results in the near future (Dr. Jan C.C., personal communication). One of the possible solutions is to create a DH protocol similar to corn, by creating a line which possesses a haploidisation inducer, a marker and the abillity to eliminate the maternal chromosome (Dr Hulke B., personal communication). Although both projects are currently in motion, it is likely that a few more years will pass before a protocol is developed to satisfy the needs of selection programs.

MUTATIONAL BREEDING

In the broadest sense, mutations are heredetary changes in genomes. The subdivision of mutations is complex - mutations, and resulting changes in gene function, can arise from a single base change (point mutation) or from structural changes to the genome (insertion/deletion, duplication, translocation, inversion, and *etc.*). They can be spontaneous or induced, lethal, sublethal or vital. For breeding purposes, without a doubt the most

important mutations are the vital ones. Induced mutations bare high significance in plant breeding. This branch is called mutational breeding. To induce mutations, both physical mutagens, such as gamma rays, or UV rays, and chemical mutagens, such as ethyl methane sulphonate (EMS) and methyl methane sulphonate (MMS), can be used. A majority of agriculturally-important induced mutations have been created using EMS. The success of mutational breeding is not guaranteed. Desirable mutations appear at very low frequencies and always with undesirable background mutations. Although a lot of work has gone into sunflower mutations, only a few are commercially applicable. The application of purposeful mutations is still in its developing stages. Chemical mutagen methodologies have been applied most frequently, likely due to the high rate of mutagenesis, and reduced rates of chromosomal aberration/restructuring. Causal mutations have been identified for important morphological traits such as plant height, leaf surface, shortening of the growth season, sterility, disease resistance/tolerance, oil content, and changes in oil quality. The frequency of induced mutation depends, not only on the agents, but also on the base genotype used in the mutation program (Gvozdenovic *et al.*, 2009).

It is highly likely that the first succesful commercialization of mutants in sunflowers was a spontaneous mutation resulting in the chrysanthemum sunflower, but still, the availability of spontaneous, commercially relevant mutations is limited. Because of this, induced mutations are widely applied in modern breeding. Many sunflower mutations have been published to date, but very few have found commercial application. One of the most well-known, and economically most significant induced mutations affects acetohydroxyacid synthase (Al khatib *et al.*, 1998). Sunflower tolerance to imidazolinones and sulfonylurea herbicide chemistries has significantly increased competitivness of sunflower in production and enabled manufacturers easier control of weed growth and, with the use of Clearfield technology, the control of broomrape. The ClearField-Plus mutation (Sala *et al.*, 2008) patented by Nidera and BASF as well as the +M7 SU trait (Gebard and Huby, 2004) patented by DuPont are widely used in breeding today. Fatty acid composition mutations have also found their commercial use. The best known and most widely used is the Pervenats mutation (Soldatov, 1976) obtained by treating the seed of VNIIMK 8931 with 0.5% dimethyl sulfate. It led to the increase in oleic acid to 80-90%. The Pervenets variety is used to obtain gene lines and hybrids of high oleic content.

One of the notable mutations impacts tocopherol content. The LG17 line manifests a gamma tocopherol content of 95% (Popov and Demurin, 1987), while the T2100 line (Velasco and Fernandez-Martinez, 2003) obtained by an EMS mutation of the Peredovik seed showed a gamma tocopherol content upwards of 85%. Sunflowers are the first plant system in which tocopherol controlling genes, known as Tph1 and Tph2, have been identified (Popov *et al.*, 1988). There are many methods to rapidly identify mutations, but TILLING (Targeting Induced Local Lesions in Genomes) can efficiently establish the existence of mutations in plant populations (McCallum *et al.*, 2000). This technique certainly has its own array of benefits: widespread application, efficacy when applied to small populations, and the ability to target any gene (Henry and Comai, 2014).

ELUCIDATING THE IMPACT OF COMBINING TABLE-STAKES AND NOVEL TRAITS USING CROP MODELING

One of the greatest challenges found in breeding today is the selection of genotypes which bare higher yields in ever more volatile and complex climate conditions. The creation of a breeding ideotype is one of the main goals required to reach maximum expression in

traits which influence, most of all, seed yield. The main paradigm is found in the efficient usage of the basic GxExM model which is little different than standard GxE because management options allow genetics to fully express their potential. An ideotype is defined as a combination of morphological and/or physiological traits optimizing crop performances to a particular biophysical environment and crop management. The adaptability of a genotype depends largely on introducing new traits into hybrids which provide better productivity and stability. In broader sense, the ideotype is a plant with ideal characteristics for a certain climate zone accounting for climate changes (Donald, 1968; Semenov and Stratonovitch, 2013).

Applied crop modeling should allow the integration of different techniques (Struick *et al.* 2007). As described by Yin and al. (2004, 2003), crop physiology should support the discovery of complex traits by taking advantage of data and knowledge integration under a Genotype by Environment approach. The final aim being transversal integration, where the link between the phenotype and the genotype would be fulfilled.

POST-GENOMIC ERA

The main goal of the Sunflower Genomic Resources Consortium (formed in 2012, a collaboration between the Genome Canada project, the University of Georgia, the French National Institute for Agricultural Research, and private partners) was development of the first “high quality genomic sequence of sunflower genotype HA412-HO”. Even with minor problems, the project has delivered, and the first bronze version of HA412-HO has been published. So what can we expect in the future? There are lots of challenges, possibilities, and benefits to be found in the sunflower post-genomic era. Using high-throughput sequencing technologies, we will enhance our ability to detect mutation, small regulatory RNA species (miRNA, siRNA, ncRNA), and we will progress towards whole transcriptome analysis. This will allow characterization and elucidation of mechanisms for gene regulation in sunflower (Aoki *et al.*, 2013) with high impacts for our scientific, agricultural, commercial aspirations.

Another post-genomic era application is gene-editing technology. The access to one or more (complete) genomic sequences and the support for cDNA and other transcribed sequences allows not only for the determination of copy numbers and splice-variants within the species itself, but also related homologs and orthologs found in other species, which can be used to infer the functions of native allele(s), as well as changes in function resulting from modifications made through editing (Ledford H. 2015). These are just a few examples. As time progresses, we are witnessing that genomic information is no longer a bottleneck or a limiting factor in sunflower.

LITERATURE

- Abdi, R. Darvishzadeh N.A., Jafari M., , Pirzad A. And Haddadi P. (2012): Genetic analysis and QTL mapping of agro-morphological traits in sunflower (*Helianthus annuus* L.) under two contrasting water treatment conditions. *Plant Omics Journal*, 5(2):149-158
- Al-Khatib, K., Baumgartner, J.R., Peterson, D.E. and Currie, R.S. (1998). Imazethapyr resistance in common sunflower (*Helianthus annuus*). *Weed Science* 46: 403-407.
- Busemeyer L. Mentrup D., Möller K., Wunder E., Alheit K., Hahn V., Maurer H.P., Reif J.C., Würschum T., Müller J., Rahe F., Ruckelshausen A. (2013). BreedVision — A

- Multi-Sensor Platform for Non-DestructiveField-Based Phenotyping in Plant Breeding. *Sensors*, 13: 2830-2847.
- Cadic E., Coque M., Vear F., Grezes-Besset B., Pauquet J., Piquemal J., Lippi, Y., Blanchard P., Romestant M., Pouilly N., Rengel D., Gouzy J., Langlade N., Mangin B., Vincour P. (2013) : *Theor Appl Genet* 126:1337–1356.
- Collard B.C.Y., Jahufer M.Z.Z., Brouwer J.B Pang.E.C.K. (2005): An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* 142: 169–196.
- Davis B.D.(1949):The Isolation of Biochemically Deficient Mutants of Bacteria by Means of Penicillin". *Proceedings of the National Academy of Sciences of the United States of America* 35 (1), 1–10.
- Donald C.M. (1968). The breeding of crop ideotypes. *Euphytica* 17: 385–403.
- Furbank R.T., Tester M. (2011): Phenomics – technologies to relieve the phenotyping bottleneck. *Trends in Plant Science*. Vol. 16 (12): 635-644.
- Gabard, J.M. and J.P. Huby. 2004. Sulfonylurea-tolerant sunflower line M7. U.S. Patent 6,822,146 B2.
- Gvozdenović, S., Bado, S., Afza, R., Jocić, S. and Mba, C. 2009. Intervarietal differences in response of sunflower (*Helianthus annuus* L.) to different mutagenic treatments. In Shu, Y.Q. (ed.) *Induced plant mutations in the genomics era*. FAO, Rome: 358-360.
- Heijden G., Yu Song Y., Horgan G., Polder G., Dieleman A., Bink M., Palloix A., Eeuwijk F., Glasbey C. (2012). SPICY: towards automated phenotyping of large pepper plants in the greenhouse. *Functional Plant Biology*, 39: 870–877
- Henry I.M. and Comai L. (2014). Rapid Mutation Detection for Crop Functional Genomics. *ISB News Report*.
- Hepp A-L., Sendon-Lopez M., Portwood D., Earll M., Scholes J.D. (2012). Metabolomic analysis of the resistance response in sunflower roots to the parasitic weed *Orobancha cumana*. Proceeding of 12th World Congress on Parasitic Plants, Sheffield,UK : p. 83
- Herve D., Fabre F., Berrios E.F., Leroux N., Al Chaarani G., Planshon C., Sarrafi A., Gentzbittel L. (2001): QTL analysys of photosynthesis and water status traits in sunflower (*Helianthus annuus*L.) under greenhouse conditions. *Journal of Experimental Botany* , 52 (362): 1857 – 1864.
- Kiani S.P.,Maury P., Nouri L., Ykhlef,N., Grieu P. and Sarrafi A.: QTL analysis of yield-related traits in sunflower under different water treatments. *Plant Breeding* 128: 363—373
- Koh Aoki K., Yoshiyuki Ogata Y., Igarashi K. , Yano K., Nagasaki H., Kaminuma E. , Toyoda A. (2013). Functional genomics of tomato in a post-genome-sequencing phase. *Breeding Science* 63: 14–20
- Ledford H. (2015): CRISPR, the disruptor. A powerful gene-editing technology is the biggest game changer to hit biology since PCR. But with its huge potential come pressing concerns. *Nature* 522, 20-24
- Mahlein A.K., Steinern U., Hillnhütter C., Dehne H.W., Oerke. E.C. (2012). Hyperspectral imaging for small-scale analysis ofsymptoms caused by different sugar beetdiseases. *Plant Methods*, 8(3): 1 -13.

- Maleki H.H., Darvishzadeh R., Ahmad S. (2014): Genetic linkage map and QTL analysis of partial resistance to black stem in sunflower (*Helianthus annuus* L.). *Australasian Plant Pathol.* 43:205–213
- McCallum C.M., Comai L., Greene E.A., Henikoff S. (2000). Targeted screening for induced mutations. *Nat Biotechnol.*, 18(4):455-7.
- Perez-de-Castro A.M., Vilanova S., Canizares J., Pascual L., Blanca J.M., Díez M.J., Prohens J. and Pico B. (2012). Application of Genomic Tools in Plant Breeding. *Current Genomics*, 13: 179-195.
- Popov, P.S., and Demurin, Y.N. (1987). Mutation variability and inheritance of tocopherol composition in sunflower seeds of VIR collection. *Breeding and genetics of technical crops*. VIR, Leningrad, 113: 30-33.
- Popov, P.S., Dyakov, A.B., Borodulina, A.A. and Demurin, Y.N. (1988). Genetic analysis of tocopherol and fatty acid composition in sunflower seeds. *Genetics* 24(3): 518-527.
- Roessner U., Bacic A. (2010): Metabolomics in plant research. *Australian Biochemist*, 20(3): 9-12
- Sala, C.A., Bulos, M. and Echarte, A.M., 2008. Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. *Crop Science*, 48: 1817-1822.
- Semenov M.A., Stratonovitch P. (2013). Designing high-yielding wheat ideotypes for a changing climate. *Food and Energy Security* 2: 185–196.
- Soldatov, K.I. (1976). Chemical mutagenesis in sunflower breeding. In: ISA (ed.) *Proceedings of the 7th International Sunflower Conference*, Krasnodar, USSR: 352-357.
- Spring O., Pfannstiel J., Klaiber I., Conrad J., Beifuß U., Apel L., Aschenbrenner A.K., Zipper R. (2015) The nonvolatile metabolome of sunflower linear glandular trichomes. *Phytochemistry*. 119:83-9.
- Steinfath M., Strehmel N., Peters R., Schauer N., Groth D., Hummel J, Steup M., Selbig J., Kopka J., Peter Geigenberger P., Joost T., Dongen J.T. (2010). Discovering plant metabolic biomarkers for phenotype prediction using an untargeted approach. *Plant Biotechnology Journal*, 8: 900–911.
- Struik PC, Cassman KG, Koornneef M. 2007. A dialogue on interdisciplinary collaboration to bridge the gap between plant genomics and crop sciences. In: Spiertz J.H.J., Struik P.C., van Laar H.H., eds. *Scale and complexity in plant systems research: gene–plant–crop relations*. The Netherlands: Springer: 319–328.
- Velasco, L., J.M. Fernandez-Martinez. 2003. Identification and genetic characterization of new sources of beta- and gamma-tocopherol in sunflower germplasm. *Helia* 26 (38):17-23.
- Wagner M.H., Demilly D., Ducournau S., Durr C., Lechappe J. (2011): Computer vision for monitoring seed germination from dry state to young seedlings. *Seed Testing International* 142: 49-51.
- Wills, D.M., Abdel-Haleem, H., S.J. Knapp, and J.M. Burke. (2010): Genetic architecture of novel traits in the Hopi sunflower. *J. Hered.* 10:1093
- Xinyou Yin X., Stam P., Kropff M.J, Schapendonk C.H.M. (2003). Crop Modeling, QTL Mapping, and Their Complementary Role in Plant Breeding. *Agronomy Journal* 95: 90–98
- Yin X., Paul C. S., Kropff M.J. (2004). Role of crop physiology in predicting gene-to-phenotype relationships. *Trends in Plant Science* 9 (9): 426-432.