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Evolution of durum wheat breeding in Italy

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Abstract. The paper reports on the scientific effort for improving durum wheat production and quality made by Italian breeders and geneticists by exploiting the rich reservoir of genetic variation present in the Mediterranean area and in the germplasm introduced from distant geographical areas, as well as in attempting to understand the genetic control of agronomically and technologically important traits.

Keywords. Durum wheat – Breeding – Genetics, Italy.

Evolution de la sélection du blé dur en Italie

Résumé. *Dans cet article, nous allons parcourir les efforts scientifiques déployés par des sélectionneurs et des généticiens italiens pour améliorer la production et la qualité du blé dur en exploitant l'important réservoir de diversité génétique qu'abrite la région méditerranéenne et le matériel génétique provenant d'autres zones géographiques éloignées. Parallèlement, nous allons essayer de comprendre le contrôle génétique de certains caractères d'intérêt agronomique et technologique.*

Mots-clés. *Blé dur – Sélection – Génétique – Italie.*

I – Introduction

Durum wheat is the main crop in Italy in terms of land area being grown on about 1.3 million ha (Table 1), although it accounts only for 20% of the cereals production and 2% of the agricultural Gross Domestic Product (GDP). It is grown mainly in the southern part of the peninsula and in the islands, with Apulia and Sicily accounting for about 50% of the durum area. The European Union (EU-27) is the first producer of durum wheat in the world with more than 10.0 million tons of grain, with Italy playing a leading role: 50% of the durum area and production (Table 2). The European Union is also the most important consumer and from middle 1990s it has become a net importer of this cereal to offset the pasta export. This context accounts for the scientific effort to improve durum wheat production and quality made by Italian breeders, exploiting the rich reservoir of genetic variation present in the Mediterranean area as well as the germplasm introduced from distant geographical areas.

II – Breeding activities

Durum wheat breeding activities began at the outset of the XX century by exploiting the genetic variation present in landraces from southern Italy, North-Africa and West Asia. Todaro (1921) released Brottu, Lachesos, Sardaresu, Strampelli (1932) the varieties Azizia 17-45, Dauno, Duro di Puglia, Tripolino, and Senatore Cappelli, De Cillis (1942) bred Russello and Timilia, and Conti (1948) selected Azizia 301 and Azizia 302, Capinera, Ricco, Rossarda, Rossello, Sardo, Triminia. Overall a set of successful varieties were still present in southern Italy and islands during '70s. In fact, a FAO mission in 1971 was able to trace them still grown in Sicily (Porceddu and Bennet, 2071), and a numerical taxonomy analysis (Bogyo and Porceddu, 1075; Porceddu et al., 1981) confirmed the classification produced by De Cillis (1927). A special mention deserves Senatore Cappelli. Released in 1915, it represented a milestone in durum wheat breeding; its cultivation

covered more than 60% of the durum wheat area for several decades (Bozzini, 1989) and it is still grown in some areas for specific purposes or specific brand end-products, such as the monovarietal pasta manufactured in Apulia and Sardinia. Cappelli was selected from the North African landrace Jean Khetifah and well delineates the "North African" plant ideotype (waxy, tall, yielding but rather late ripening, good quality). For decades it has been crossed to "Syrian" types (no-waxy, shorter and early ripening but prone to lodging, low quality) and to other genetic material, including bread wheat, to endow the new releases with earliness, yield stability and quality across environments and years.

Several varieties, such as Garigliano (Strampelli, 1932), Capeiti, Patrizio (Casale, 1955), Grifoni, Appulo (Grifoni, 1964), Karel, Maristella (Barbieri and Deidda, 1968), and Trinakria (Ballatore, 1970) were released in the 1930-1970 period. They were characterized by good yielding ability, good quality, and adaptability to the southern Italy pedo-climatic conditions. An additional variety, ISA-1, selected in Apulia by Dionigi (1971), was characterized by earliness and thus able to escape to drought stress, although its yielding ability and quality did not meet the farmers' preference. Overall, these varieties moved yield from 1.0-1.2 to approximately 1.8-2.0 t/ha; however, despite this 80% increase, durum wheat yield was well below that of common wheat. Consequently, starting from '70s, yield increase became the main target of many breeding programmes, to be achieved by means of the introgression of useful traits from the hexaploid and wild wheat species or by mutagenesis programmes, fostering the breeding strategy adopted by breeders after world war II in selecting for reduced number of tillers per plant, increased number of spikes per unit of land, short stature plants, able to exploit higher fertiliser inputs and improved harvest index. Instrumental in this endeavour were the crosses between Italian genetic resources and short stature bread wheat, such as the Japanese red wheat Aka komugi, already used by Strampelli in bread wheat breeding. Thus by means of introgression from common wheat and selection for high spikelet fertility, Maliani (1968) obtained the cvs. Viscardo Montanari, Carlo Jucci, Giovanni Raineri.

An additional push in this direction was produced by D'Amato, Scarascia, Bozzini, Bagnara, Rossi and Mosconi, that, while studying the effects of radioactive mutagens on plants at the CNEN Casaccia Research Centre, were able to isolate some short statured lodging resistant, early ripening lines, four of which were released as finished varieties under the name of Castelporziano and Castelfusano, obtained from Cappelli, Casteldelmonte from Grifoni, and Castelnuovo from Garigliano (Scarascia Mugnozza, 1968; Bogyo *et al*., 1969; Bozzini and Girotto, 1971). Thanks to its erectoid gene, Castelporziano was able to stand in the most adverse lodging conditions. Stranger enough the molecular mechanism of the erectoid gene has yet to be clarified. Selection of segregating lines from a cross between one of those mutant lines (CpB144) and a dwarf CIMMYT line produced Creso, a high yielding variety, good quality, well adapted to different environmental conditions, almost filling the gap between durum and common wheat. In fact, in some environments Creso yield reached 10 t/ha and for a number of years it had more than 60% share in durum wheat land area (Bozzini *et al*., 1984; Bozzini, 1985). The attempt to reduce plant height by using CIMMYT lines carrying the Norin 10 dwarfing gene *Rht1* on chromosome 4A*,* in which a point mutation has produced a stop codon (the substitution of T with C converts the CGA codon for alanine in the TGA stop codon), was already in use at the Experimental Institute of Cereal Research by Vallega and Zitelli. They succeeded in selecting dwarf, disease resistant lines, which were later released as varieties having the prefix VAL- in their name (Zitelli and Vallega, 1968; Zitelli, 1973). The use of CIMMYT lines not only produced dwarf, lodging resistant plants, but also with a higher spikelet fertility and therefore a higher number of seeds per spike.

Table 1. Area (hectars) and production (metric tons) of durum wheat cultivated in individual regions of Italy during the 2011-2013 period.

Regions	2011		2012		2013		Averages	
	Ha	T	Ha	T	Ha	T	Ha	T
Piemonte	3,362	14,995	1,146	2,670	895	3,989	1,801	7,218
Valle d'Aosta	3	8	2	6	-	-	3	7
Lombardia	8,653	45,175	9,124	55,145	7,997	38,370	8,591	46,230
Veneto	8,110	45,225	7,676	50,492	3,859	24,734	6,548	39,995
Friuli-Ven. Giulia	763	3,269	741	3,180	-	-	752	3,225
Emilia-Romagna	41,993	252,438	47,388	287,928	43,388	267,077	44,256	269,148
Toscana	74,918	248,532	92,117	292,176	61,279	191,526	76,012	244,078
Umbria	18,005	100,995	18,000	105,487	17,355	99,525	17,786	102,002
Marche	120,380	479,819	132,350	606,711	123,604	491,855	125,444	525,561
Lazio	45,441	161,415	77,450	-	41,600	125,200	54,830	168,528
Abruzzo	29,860	111,015	34,083	130,161	32,240	133,846	32,061	124,570
Molise	50,766	153,819	61,500	172,200	59,600	166,880	57,288	164,300
Campania	55,239	144,134	55,317	188,212	59,609	189,622	56,721	173,317
Puglia	272,750	813,430	274,700	750,810	349,500	1,131,300	298,980	888,893
Basilicata	117,350	344,550	82,113	334,310	116,943	327,008	105,468	335,289
Calabria	23,537	58,861	31,037	80,195	31,537	91,814	28,703	76,286
Sicilia	295,690	818,314	301,641	872,287	287,331	800,690	294,887	818,235
Sardegna	32,154	62,490	34,036	82,084	34,514	74,935	33,568	73,169
North	62,884	361,109	66,077	399,420	56,139	334,169	61,700	364,899
Center	258,744	990,760	319,917	1,233,398	243,838	908,106	274,166	1,044,088
South	877,346	2,506,612	871,581	2,610,258	971,274	2,916,093	906,734	2,677,654
Italy	1,198,974	3,858,481	1,257,575	4,239,426	1,271,251	4,158,369	1,243,456	4,058,973

Source: ISTAT.

Table 2. World production of durum wheat (million tons) during the 2009-2013 period.

Country	2009	2010	2011	2012	2013	Average
EU-27	10.0	8.7	9.1	8.2	7.9	8.8
France	2.1	2.1	2.5	2.1	2.4	2.2
Greece	1.1	1.3	1.3	0.9	0.7	1.1
Italy	5.2	3.6	4.1	3.9	4.2	4.2
Spain	1.1	1.4	0.9	0.9	0.4	0.9
Kazakhstan	2.5	2.6	1.7	3.0	1.4	2.2
Canada	5.5	5.4	3.0	4.2	4.6	4.5
Mexico	2.0	2.2	2.2	2.2	2.1	2.1
USA	2.3	3.0	2.9	1.4	2.2	2.4
Argentina	0.2	0.2	0.3	0.2	0.2	0.2
Syria	1.2	1.8	1.6	1.7	1.5	1.6
Turkey	3.0	3.1	2.9	3.0	3.0	3.0
India	1.1	1.0	1.0	1.1	1.2	1.1
Algeria	0.9	2.9	2.2	2.5	3.0	2.3
Libya	0.1	0.1	0.1	0.1	0.1	0.1
Morocco	1.0	1.9	1.6	1.7	1.3	1.5
Tunisia	1.4	1.4	0.6	1.2	1.3	1.2
Australia	0.5	0.5	0.5	0.6	0.5	0.5
Others	7.2	6.2	5.3	5.7	5.1	5.9
WORLD TOTAL	38.9	41.0	35.0	36.8	35.4	37.4

Source: IGC – CWB.

The following years were characterized by intense breeding programmes, with CIMMYT materials included in almost every cross as partner of Italian germplasm. This allowed the release of a

number of high yielding cultivars by the Experimental Institute of Cereal Research (Foggia section) and by private seed companies, such as Società Produttori Sementi (Bologna), Società Italiana Sementi (Bologna), Eurogen (Enna), ISEA (Ancona) and COSEME (Foggia). The number of registered varieties, which was lower than 10 at the beginning of 1980s, grew up tremendously (6 until 1980, 19 in the decade 1981-1990, 62 in 1991-2000, and 145 in 2001-2013), thanks also to the EU financial support for durum wheat linked to the use of registered variety seed and to the introduction in Italy of the National Variety Register. In 2013, the National Seed Certifying Agency, ENSE, certified approximately 203,585 t of durum wheat seed (Table 3), with Iride having the highest share (12.4%), followed by Simeto and Saragolla with 11.4% and 9.3%, respectively; 76.1% of certified seed was interested by 20 cultivars, out of 136 registered varieties. Creso, 35 years old, was still grown on 1.3% of the durum wheat land devoted to certified seed.

Table 3. Certified seed (t) of the top twenty durum cultivars grown in Italy in 2013.

Cultivar	Certified seed		Cultivar	Certified seed	
	(t)	(%)		(t)	(%)
Iride	25,310	12.4	Anco Marzio	3,993	2.0
Simeto	23,241	11.4	Achille	3,936	1.9
Saragolla	18,987	9.3	Tirex	3,593	1.8
Core	12,175	6.0	San Carlo	2,507	1.7
Claudio	11,960	5.9	Pietrafitta	3,492	1.7
Quadrato	7,453	3.7	Dylan	3,483	1.7
Duilio	6,971	3.4	Svevo	2,675	1.3
Levante	5,968	2.9	Creso	2,646	1.3
Orobel	4,814	2.4	Miradoux	2,164	1.1
Rusticano	4,454	2.2	Other cvs	48,659	23.0
Arcangelo	4,104	2.0	TOTAL	203,585	100.0

An analysis of a Mediterranean collection of varieties by means of molecular markers, four per chromosome (Maccaferri *et al*., 2005), shed some light in the history of the last 50 years of breeding activity and strategic approaches followed by different breeding groups, and allowed to indicate some important points: 1) the main Italian durum cultivars, with the exception of Svevo, Neodur and Rusticano, have Cappelli in their pedigree, indicating that the ideotype preferred by Strampelli is somehow still valid, with the exception of plant height; 2) breeding has not added genes one by one but rather has restructured the entire genome; 3) breeding has preserved entire gene blocks for a long period, whereas more recently has produced a fine restructuring.

III – Selection targets

As far as the selection targets are concerned, lodging resistance has been already stressed as one of the most important targets, followed by earliness, limited number and simultaneously flowering fertile tillers, and number of fertile flowers per spikelet. Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century was recently investigated by De Vita *et al*. (2007) who showed that differences in agronomic traits are generally similar to differences observed in hexaploid wheat, with an annual genetic yield gain of 19.9 kg ha^{-1} $year^{-1}$. The genetic gain was most clearly associated with a higher kernels number m^{-2} indicating a larger grain-sink size and a higher number of spikes m^{-2}. The gradual reduction in plant height associated with an increased harvest index has represented the main breeding goal with an effect on the sink capacity and on the biomass partitioning.

1. Genetic resistance

About twenty pathogens and five insects can undermine wheat yield. The breeders prevalent attitude was to prefer low levels of long-lasting resistances, based on several genes, instead of high level of resistance controlled by single genes, in spite of the wide genetic variability present in cultivars and wild populations of the related species.

Stem rust (*Puccinia graminis* f. sp. *tritici*) has caused the heaviest losses in durum wheat. The first sources of resistance studied were a group of wheats from North Dakota (Yuma, Ld 390, Lakota and Wells), which were known to carry resistant factors derived from durum wheat, and some accessions of ssp. *dicoccum* (Zitelli, 1968), and were of great importance in controlling the pathogen's races existing in Italy (Bozzini, 1966; Zitelli, 1973). The sources of resistance to leaf rust, *Puccinia recondita*, were all found with poor agronomic performance. Using lines derived from Ld 390, Beladi 116, Tremez molle, Kyperunda and Gaza, Zitelli (1973) was able to transfer resistance to leaf rust into Italian varieties, thereby obtaining the Giorgio and Gerardo breeding lines used for further work by several durum breeders. The first source of resistance factor to powdery mildew (*Blumeria graminis*) was Yuma, whose resistance factors derived from ssp. *dicoccum* cvs Vernal and Khapli (Bozzini, 1966; Zitelli and Vallega, 1968; Zitelli, 1972). Later, a number of resistance genes were identified and mapped by using molecular markers, such as the novel resistance gene to mildew, *Pm36,* identified in one accession of ssp. *dicoccoides* and mapped on the chromosome bin 5BL6-0.29-0.76; the 244 bp allele of the EST-SSR marker BJ261635 can be used for marker-assisted selection during the wheat resistance breeding process for facilitating gene pyramiding. Later, many resistance genes for rust, mildew and *Fusarium* have been identified in related species and introduced in durum wheat (see recent review by Ceoloni *et al*., 2014). Interestingly, the Italian durum wheat cultivar Creso possess a high level of durable resistance to leaf rust (*Puccinia triticina* Eriks.) based on both hypersensitive and non-hypersensitive components. In order to investigate the genetic basis of this resistance, a segregating population composed of 123 recombinant inbred lines (RILs) derived from the cross Creso × Pedroso, was evaluated for disease severity in adult plants under field conditions (Marone *et al*., 2009). Besides some minor QTLs, one major QTL explaining both reduction of disease severity in the field and increased latency period was found on the long arm of chromosome 7B, and closely associated PCR-based and DArT markers were identified. Association mapping on a germplasm collection of 164 elite durum wheat accessions confirmed the presence of the *Lr14* resistance gene on 7BL in the cultivars Llareta and Creso (Maccaferri *et al*., 2010). *Lr14* can be considered as an important gene for resistance to leaf rust currently exploited by durum breeders in the Mediterranean areas.

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is one of the most important diseases of wheat worldwide, resulting in yield losses and mycotoxin contamination. Transgenic wheat plants expressing the bean PvPGIP2 (polygalacturonase-inhibiting proteins) in their flowers were found to have a significant reduction of symptoms when infected with *F. graminearum* (Ferrari *et al*., 2012) and suggest that polygalacturonases (PGs) likely play a role in of floral tissues infection, and that PGIPs incorporated into wheat may be important for increased resistance to FHB. Cereals contain xylanase inhibitor (XI) proteins which inhibit microbial xylanases and are considered part of the defense mechanisms to counteract microbial pathogens. A number of transgenic plants constitutively overexpressing TAXI-III, a member of the TAXI type XI that is induced by pathogen infection, were produced by Moscetti *et al*. (2013) and results showed that TAXI-III endows the transgenic wheat with new inhibition capacities which correlate with a significant delay of Fusarium head blight disease symptoms. These results provide clear evidence in planta that XI are involved in plant defense against fungal pathogens and show the potential to manipulate TAXI-III accumulation to improve wheat resistance against *F. graminearum*.

2. Drought stress

Drought is by far the most important factor affecting production and production stability; it has been therefore a classical topic in durum research. A number of morphological and physiological indices have been proposed for the selection of tolerant lines, such as stomata form, dimensions, and number per unit of leaf area, leaf water potential, osmotic adjustment ability, relative moisture content, accumulation of osmolites and abscissic acid (Tuberosa and Salvi, 2002; Cattivelli *et al*., 2008; Tuberosa, 2012). The best test, although empirical, is provided by the evaluation of lines in normal and stress conditions, and computing methods for establishing the different environmental conditions have been proposed. It is important to underline that phenotyping continues to represent the key step in understanding stress tolerance in the molecular era.

Gene cloning has opened new prospects in elucidating plant mechanisms elicited by stress and has led to the isolation of genes whose activity is controlled by stress events, although their precise function is still to be defined. Particularly interesting in this issue are results related to the expression of sequences and mRNA in plants of Capeiti and Creso, tested under different levels of water stress at the flowering stage (Cattivelli *et al*., 2002). A genomic map of major loci and QTLs affecting stress tolerance identified the crucial role of the group 5 chromosomes, where the highest concentration of QTLs and major loci controlling plant's adaptation to the environment (heading date, frost and salt tolerance) has been found. Extensive molecular studies have led to the cloning of many stress-related genes and responsive elements. The expression of some stress-related genes was shown to be linked to stress-tolerant QTLs, suggesting that these genes may represent the molecular basis of stress tolerance (Aprile *et al*., 2013).

Nine populations of durum wheat, from three environmentally contrasting regions of Ethiopia (Tigray, Gonder and Shewa), were analyzed by SSRs markers in order to verify the presence of LD and to detect loci with reduced variation, possibly as consequence of selective sweeps. Results indicated the existence of high linkage disequilibrium among loci and the presence of some selective sweeps in chromosome 4A sequences, close to loci (QTL) previously identified as related to drought tolerance (Pagnotta *et al*., 2007). Genes from the DREB family are involved in plant's responses to dehydration and possibly play a role in their ability to tolerate water stress. The isolation and characterization of a gene in durum wheat, namely TdDRF1, which belongs to the DREB gene family and produces three forms of transcripts through alternative splicing, has been reported (Latini *et al*., 2007). Recently, many transcription factors for tolerance to salt and drought stresses have been identified, and the multi-alignments of conserved domains in DREB1, WRKY1 transcription factors (TFs), and HKT-1 has allowed to identify functional single nucleotide polymorphisms (SNPs) (Mondini *et al*., 2012). All the discovered mutations were able to generate changes in amino acid sequences of the corresponding proteins. Most of the identified SNPs were found in salt and drought tolerant durum wheat genotypes. A different stress responsive strategy was found in two durum wheat cultivars characterized by different water use efficiency, subjected to drought, heat and a combination of both stresses (Rampino *et al*., 2012): the cv Ofanto (lower water use efficiency) activated a large set of well-known drought-related genes after drought treatment, while Cappelli (higher water use efficiency) showed the constitutive expression of several genes induced by drought in Ofanto and a modulation of a limited number of genes in response to stress.

A review of breeding progresses on drought stress tolerance (Cattivelli *et al*., 2008) pointed out that selection for high yield in stress-free conditions has indirectly improved yield in many water-limiting conditions. To reduce the gap between yield potential and actual yield in drought-prone environments, three main approaches can now be exploited: (i) plant physiology has provided new insights and developed new tools to understand the complex network of drought-related traits; (ii) molecular genetics has detected many QTLs affecting yield under drought or the expression of drought tolerance-related traits; (iii) molecular biology has provided genes useful either as candidate sequences to dissect QTLs or for a transgenic approach. The extent of information,

that breeders currently have, offers new tools for breeding, such as markers for QTLs and single genes for plant transformation. This strategy will lead to new cultivars with high yield potential and high yield stability, that in turn will result in superior performance in dry environments.

3. Grain and pasta quality

Durum wheat grain is today essentially used in pasta manufacturing in Italy and pasta quality has constantly been one of the most important breeding objectives. In southern Italy durum grain is also utilised in typical bread preparation, such as the well-known “Pane di Altamura”. De Cillis (1942) was the first to show that pasta produced by using vitreous grains, which possess higher protein content, has better cooking quality than that obtained from starchy grains, which possess lower protein content. The same conclusions were reached, years later, by many other scientists who also showed the existence of a consistent relationship between gluten properties and content and pasta cooking quality (Novaro *et al*., 1993; Mariani *et al*., 1995; Autran and Galterio. 1989; D’Egidio *et al*., 1990). These findings promoted a wide array of studies on grain protein composition, grain protein content and grain colour, to which Italian scientists participated actively using different approaches.

4. Grain protein composition

Pasta quality is strongly depending from gluten quantity and quality. Gluten is a proteic complex composed of gliadin and glutenin components confering viscoelasticity and plasticity to gluten mass, respectively. Since gluten was shown to be composed by polypeptides controlled by single co-dominant factors, specific attention has been deserved to the analyses of these genes (Dal Belin Beruffo *et al*., 1981, 1982; Porceddu *et al*., 1983; Lafiandra *et a*l., 1984, 1987, 1990). It was thus possible to demonstrate that: a) gliadins components are coded by genes *(Gli-1)* located on short arms of homoeologous chromosomes of groups 1 and 6 (Lafiandra *et al*., 1984); b) loci for low molecular weight (LMW) glutenin subunits (*Glu-3*) are located on short arms of group 1 chromosomes closely linked to *Gli-1* (Pogna *et al*.,1990); c) a tremendous amount of genetic variation exists for gliadin and glutenin subunits, both in cultivated wheats and wild relatives (Ciaffi *et al*., 1992) offering opportunity for selection. In fact a number of breeding lines has been selected for the presence of different gliadin and glutenin components. Related to these aspects, many studies have been carried out on the allelic variability of *Gli* and *Glu* loci and on the relationships with technological quality. Pogna *et al*. (1988), utilising a recombinant line containing ɣ-42 gliadin and LMW-2, firstly reported by Margiotta *et al*. (1987), were able to show the functional direct role of LMW subunits in gluten viscoelastic properties. Pogna *et al*. (1990) reported that genes different from those at *Glu* loci could be involved; in fact negative correlations between proteolitic activity and cooking quality was found (Dal Bellin Peruffo and Pallavicini, 1981; Petruzzelli *et al*., 1981). The total content of the glutenin components, the presence of specific HMW-GS and of specific gliadin subunits has been directly associated with the higher rheological characteristics of gluten. These proteins include the γ-45 gliadin and various ϖ and β gliadins coded by the Gli-*B1 l*ocus localized on the short arm of chromosome 1B and genetically associated to the *Glu-B3* locus coding for a group of LMW-GS, named LMW-2. Wheats with the γ-45 subunit have six different alleles at *Glu-B3,* and this explain the wide variability of grain quality (from average to high) of durum lines all presenting the γ-45 gliadin component. The γ-45 band represents, actually, a quality marker since *Gli-B1* is closely associated to the *Glu-B3* locus. Indeed, gluten quality essentially depends on specific glutenin subunits coded by *Glu-A3, Glu-B3* and *Glu-B2* loci. Protein bands, visualized after electrophoresis on poliacrylammide gel, are widely used as biochemical markers of grain quality in modern breeding programs.

These findings promoted additional studies, such as those on the analysis of a family of nine genes, located on group 4 chromosomes, coding for protein disulfide isomerase (PDI), which has a redox role and may affect protein folding and assembling (Ciaffi *et al*., 1999; Ciaffi *et al*., 2001);

PDI genes have been cloned and sequenced (Ciaffi *et al*., 2006) and transgenic plants have been produced in cooperation with CIMMYT (D'Aloisio *et al*., 2010; Paolacci *et al*., 2011; Ciaffi *et al*., 2013).

Moreover, Resmini and Pagani (1981) observed that differences in the semolina and spaghetti protein matrix could promote possible interactions not only among different protein types but also between proteins and carbohydrates in determining pasta quality. However, there were differences depending on whether semolina or dry pasta was used in the analyses. When semolina was used, the role of proteins was prevalent and that of other components was irrelevant. When pasta was used, the dominance of proteins decreased and the role of starch increased and was positive; amylopectin behaves in the same manner as starch, and starch change at the expenses of amylose is to be preferred (D'Egidio *et al*., 1979). Quite similar results were obtained with pentosans, which, as known, consist of highly branched linear xylanes. Medcalf *et al.* (1968) showed that water soluble pentosans from durum wheat are more branched than those from hard red spring wheat, and even small differences in the branching degree may greatly alter the degree and type of interactions of polysaccharides with proteins. The yield of water soluble pentosans from spaghetti is much higher than that from semolina, whereas the opposite is true for the water insoluble ones (Lintas and D'Apollonia, 1973) supporting the occurrence of differences in starch gelatinisation.

Manipulation of starch composition in cereals, and particularly in wheat, is receiving increasing attention due to recognition of its important role in food and nonfood applications. The amylose/amylopectin ratio influences the physicochemical properties of starches and nutritional value of derived end-products (Lafiandra *et al*., 2013). Identification of the key enzymes involved in the starch biosynthetic pathway has opened new avenues for altering the amylose and amylopectin ratio in durum and bread wheat. The granule bound starch synthases (GBSSI), or waxy proteins, are the enzymes responsible for amylose synthesis in storage tissues; amylopectin is produced by the concerted action of different enzymes, including starch synthases (SS), branching (SBE), and debranching enzymes (DBE). By altering the level of key enzymes involved in the regulation of starch synthesis, it is possible to generate novel starches with unique functional properties. In this respect, both low and high amylose starches are particularly interesting because they are associated with industrial and processing properties as well as with human health and nutrition (Lafiandra *et al*., 2010). The characterization of waxy genes that modify the relative amount of amylose and amylopectine was reported by Monari *et al.* (2005) and NIL have been produced and tested (Jonnala *et al*., 2010).

The remarkable innovations in pasta production processes, in particular drying technologies based firstly on low temperatures (40 - 50° C) and later raised to 60-70°C or even to 80°C, have allowed the production of pasta with an acceptable or good cooking strength by using poor quality raw materials (De Stefanis and Sgrulletta, 1990). High temperatures have been quickly adopted from pasta industry not only for baking quality improvement but also for the higher healthy conditions and the reduced times of drying. However, also with these new technologies, protein content remain a parameter of primary importance for the production of higher quality pasta (Novaro *et al*., 1993).

Durum wheat is traditionally used for the production of numerous types of pasta; however, significant amounts are also used for bread-making, particularly in southern Italy. The glutenin subunits 1Dx5 and 1Dy10, encoded by the *Glu-D1* locus on chromosome 1D in bread wheats, are positively correlated with higher dough strength. Transgenic plants for glutenin subunits have been obtained at Experimental Institute for Cereal Research (in cooperation with University of Bristol) (Terzi *et al*., 2005) and University of Bari (in cooperation with USDA, Albany) (Gadaleta *et al*., 2008). In order to study the effects of stable expression of the 1Dx5 and 1Dy10 glutenin subunits in different wheat genotypes, four durum cultivars commonly grown in the Mediterranean area (Svevo, Creso, Varano and Latino) were co-transformed, via particle bombardment of

cultured immature embryos, with the two wheat genes *Glu-D1-1d* and *Glu-D1-2b* (Gadaleta *et al*. 2008). Small-scale quality tests showed that accumulation of Dx5, Dy10 or both in transgenic durum seeds resulted in doughs with stronger mixing characteristics. Sissons *et al*. (2013) studied the effect on technological properties of pasta and bread made from durum wheat cv. Svevo and two isogenic genotypes carrying pairs of additional subunits 5+10 (S 5+10) or 2+12 (S 2+12), normally present in bread wheat. The dough properties of the S 5+10 line were markedly different from Svevo, having over-strong, stable dough, low wet gluten and elasticity; S 2+12 also displayed stronger dough. Pasta prepared from these genotypes showed lower cooked firmness. Bread loaf volume and loaf score decreased as more bakers flour was replaced by durum flour, but the decline varied with the genetic material and dosage. The greatest reduction in loaf volume occurred using S 5+10 and the least with S 2+12, which was similar to Svevo. Bake score was reduced with S 5+10 only. These work show that it is possible to manipulate the processing properties of pasta and durum-bread-wheat blends by altering the glutenin subunit composition.

5. Grain protein concentration

Protein content and other qualitative parameters of durum wheat grain are polygenic characters strongly influenced by environmental factors. Their evaluation and the obtainment of improved lines are expensive, laborious and time-consuming because of the low heritability and of the complex biological bases. Segregant off-springs and lines have to be evaluated in different environments in order to obtain reliable data and the identification of superior genotypes. Such evaluations sometimes require an elevated amount of grain. For these reasons, breeders are looking for alternative strategies, quicker and more reliable of conventional ones. The mapping of loci for quantitative characters allows the identification of associated molecular markers that can be used in assisted selection and, therefore, to perform a genotypic selection in alternative to the conventional phenotypic selection. In durum wheat a segregating population of recombinant inbred lines has been obtained from crossing the cv. Messapia and an accession of *dicoccoides* with high protein content; for this population a molecular map that now comprises 458 markers comprehensive of morphologic, biochemical, RFLP, AFLP and microsatellites markers has been produced (Blanco *et al*., 1998; Blanco et al, 2004). The analysis of the segregant population in eight different environments has allowed to map seven different QTLs for protein content on six chromosome arms and to clear the bases of the negative correlation between protein content and productivity observed in all cereals (Blanco *et al*., 2006). Such correlation is generally attributed to environmental factors, to nitrogen dilution in the kernels, to a higher amount of carbohydrates, to the higher energetic demands for protein synthesis with respect to carbohydrates, to genetic components. Six of the seven QTLs for the high protein content had pleiotropic effects or they were associated to QTLs for low productivity. These results are obviously important in assisted selection programs where the use of markers for the desired character should not have negative consequences on other correlated agronomic traits. In a recent study carried out on a RIL segregating population derived from crossing two commercial elite cultivars (Svevo e Ciccio), 10 independent genomic regions involved in the expression of GPC were detected, six of which were associated with QTL for one or more grain yield components (Blanco *et al*. 2012). QTL alleles with increased GPC effects were associated with QTL alleles with decreased effects on one or more yield component traits, or vice versa (i.e. the allelic effects were in opposite direction). Four QTL for GPC showed always significant effects, and these QTLs should represent genes that influence GPC independently from variation in the yield components. Such genes are of special interest in wheat breeding since they would allow an increase in GPC without a concomitant decrease in grain yield.

6. Grain colour

Yellow pigment concentration (YPC) in durum wheat is an important criterion for the assessment of semolina quality, particularly in determining the commercial and nutritional quality of end-

products. The pigment content has been taken into serious consideration only recently from Italian breeders. Yellow colour depends on several factors: carotenoid content of kernels, residual content of pigments after grain or semolina-bran conservation, the grinding rate, the oxidative degradation of enzymes, like lipoxigenases (LOX), and the conditions of pasta-making process. Genetic variability of YPC and carotenoid components was analysed in 102 wild and cultivated tetraploid wheat accessions (Di Gesù *et al*., 2009). Overall, modern cultivars showed significantly higher values of YPC compared to old cultivars and wild *dicoccum* and *dicoccoides* accessions. Lutein was the main component of carotenoids, followed by zeaxanthin and β-carotene; α-carotene and β-cryptoxanthin were minor components. Pigment concentration was negatively correlated with kernel weight and grain protein concentration; significant positive correlations were found between the yellow index *b** and YPC. The value of 4,2 ppm is considered the minimal amount in order to obtain pasta of acceptable colour. The majority of the recent French varieties and some of the Italian ones, like Grecale and Svevo exceed this value. The total carotenoid content is a polygenic character with high heritability. The isolation of BAC clones containing genes coding for three different enzymes of the carotenoid biosynthesis pathway: phytoene synthase (PSY), phytoene desaturase (PDS), and carotene desaturase (ZDS) was reported by Cenci *et al*. (2004). Primers were designed on the basis of wheat ESTs similar to the sequences of these three genes in other species, and used to screen a durum wheat BAC library (Cenci *et al*., 2003). PSY clones were localized on chromosomes 5A and 5B, PDS on chromosomes 4A and 4B, and ZDS on chromosomes 2A and 2B. Recently, 150 SSR and EST-SSR markers and 345 DArT markers, were used to construct the linkage map Latino x Primadur for subsequent carotenoid components QTL analysis (Blanco *et al*., 2011). Clusters of QTLs for total and/or one or more carotenoid compounds were detected on the same chromosome regions (2A, 3B, 5A and 7A) where QTLs for yellow pigment concentration and yellow index were identified. The molecular markers associated to major QTL would be useful for marker-assisted selection programs to facilitate high carotenoid concentration with high nutritional carotenoid compounds in wheat grain.

During pasta processing, oxidative degradation of carotenoid pigments occurs mainly due to lipoxygenase enzymes (LOX). In durum wheat, two *Lpx-1* genes have been identified on chromosome 4B, and evidences have been reported that the deletion of *Lpx-B1*.1 is associated with a strong reduction in LOX activity in semolina. The *Lpx-B1* gene family was characterized in a durum germplasm collection and showed that all of the genotypes have one of the three *Lpx-B1.1* alleles, associated with either *Lpx-B1.2* or *Lpx-B1.3*, and accounts for most of the total LOX activity in the mature grains (Verlotta *et al*., 2010; De Simone *et al*., 2010). Information on these *Lpx-B1* haplotypes provides significant improvement for prediction of LOX-1 activity levels in mature grains, and will therefore help in breeding programs aimed at selection of new durum genotypes with higher carotenoid contents in their end-products.

IV – Future perspectives

The selection of new and better genotypes of durum wheat can be obtained by several breeding strategies, all based, however, on the availability of genetic variability. The classical way consists in crossing parental lines selected for higher phenotypic traits (productivity, production stability, tolerance to drought, nutrients using efficiency, above all nitrogen, resistance to the main pathogens, etc.) and the subsequent evaluation of segregating off-springs through the pedigree method or other conventional methodology. With this approach, the possibility of selecting improved lines depends on the choice of the parental lines, especially on their genetic distance and, therefore, from the possibility to obtain a high number of recombinants. The wheat breeder must, therefore, select a combination of genes useful for a superior productive and qualitative performance of the new cultivar. However, to arrange desired genes in a single plant is not easy and it is a long-lasting and laborious process, in particular considering that, with the classic

methodologies of genetic improvement, the available genetic variability is that enclosed between individuals of the same or closely related species.

The molecular markers-assisted selection (MAS), as an approach for the identification of genotypes having certain QTLs, can be a valid instrument in order to accelerate the procedures and to reduce, therefore, the time necessary for the identification of superior genotypes (Collard and Mackill 2008). MAS uses molecular markers in linkage disequilibrium (LD) with the useful genes. However, agronomical important traits are complex and affected by many genes, each with small effect. Classical marker-assisted selection has been ineffective for such quantitative traits. The dramatic drop of the cost of DNA markers should accelerate the obtainment of crop varieties with improved yield and yield stability, quality, disease resistances and drought stress tolerance. The introduction of genomic selection (GS) is a new approach for improving quantitative traits in large crops breeding populations that uses whole genome molecular markers. Genomic prediction combines marker data with phenotypic and pedigree data to increase the prediction accuracy of breeding and genotypic values. Selection can be based on GS predictions, potentially leading to more rapid and lower cost gains from breeding (Bernardo and Yu, 2007; Goddard. and Hayes, 2007; Heffner *et al*., 2009).

The use of mutagenesis can be still used in order to increase the genetic variability, in particular for the identification of new useful characters. Thus, recently, at the Cereal Research Institute (Foggia) an experiment of chemical mutagenesis has been carried out and interesting mutants have been isolated, such as the “stay-green” that shows an extended photosynthetic activity, or other mutants characterized by elevated concentrations of K+ ions in the culm, resistance to salinity, elevated metabolic efficiency (Rascio *et al*., 2007). The reverse-genetics approaches are becoming appealing thanks to the improvement in high-throughput DNA screening techniques and the increasing number of available gene sequences. TILLING (Targeting Induced Local Lesions In Genomes; McCallum *et al*., 2000) is a significant and emerging reverse-genetics strategy that combines standard chemical mutagenesis with high-throughput techniques to screen and to identify induced point mutations in candidate genes. TILLING has been proposed as an innovative approach to generate and detect novel allelic variants starting from known genes of interest, as well as phenotypes suitable for breeding purposes. The seed company Società Produttori Sementi S.p.A. (PSB; Bologna, Italy) and the Department of Science and Technology for Agriculture, Forest, Nature and Energy (DAFNE) of Tuscia University (Viterbo, Italy) have developed a durum TILLING population of 2601 M_3 families from cv. Svevo using ethyl methanesulfonate as a chemical mutagen (Bovina *et al*., 2013). Despite the polyploid nature of the wheat genome, a preliminarily phenotypic screening of the entire M_3 population in a field-grown experiment showed a high frequency of morphological alterations (~22%). Furthermore, a reverse-genetics experiment was performed on DNA collected from M_2 leaves for the homoeologous genes *SBEIIa-A* and *SBEIIa-B* involved in starch metabolism and one non-sense mutation for both genes was identified.

In alternative to crossing, the transgene technology can be a powerful tool for widening the genetic variability and for the production of new lines. Currently, recombinant DNA technology supplies advanced instruments for the identification and the isolation of genes encoding specific characteristics in a determined organism and to transfer copies of these genes in a completely different organism. Recombinant DNA technology has been applied also in durum wheat for the production of new lines without using the classic methodologies of crossing and selection. The collaboration between the Cereal Research Institute (Foggia) and the Department of Agricultural Sciences of the University of Bristol (UK), has allowed to optimize the technique of transformation in the durum wheat (species well-known to be recalcitrant to transformation) and to obtain plants of the cultivar Ofanto transformed for glutenin subunits (Terzi *et al*., 2005). Similar results have been obtained through a collaboration between the section of Genetics and Plant Breeding of the DiSSPA (University of Bari) and the USDA of Albany (USA) that has allowed to obtain plants of three different durum cultivars (Creso, Svevo, Varano) transformed for the 5+10 HMW-

GS, normally present on the D genome of common wheat (Gadaleta *et al*., 2008). Moreover, studies are performed with the objective to dissipate doubts and perplexity about GMO in agriculture, above all those linked to the use of particular genetic markers. It is well known that the development of transgenic plants requires the use of selectable marker genes, as the efficiency of plant transformation is less than optimal for many important species, especially for monocots such as durum wheat. Many concerns have been expressed about the persistence of currently used marker genes in plants used for field cultivation. To sustain further progress in this area, alternative efficient selection methods are desirable. The 'selection efficiency' of a commonly used negative selection method that employs the *bar* gene to confer resistance to the herbicide bialaphos was compared to a positive selection employing the phosphomannose isomerase (*pmi*) gene as the selectable gene and mannose as the selective agent (Gadaleta *et al*., 2006). The selection efficiency was higher when *pmi* was used as the selectable marker gene (90.1%) than when bar was used (26.4%). Thus, an efficient selection method for durum transformation was established that obviates the use of herbicide résistance genes. At the same time, the "gene clean' technique has been setting up to combine biolistic transformation by minimal gene cassettes with genetic segregation to make marker-free transgenic wheat plants with new traits (Gadaleta *et al*., 2008).

Useful instruments in cultivated plants are the "BAC libraries" that allow cloning of large DNA fragments and generally are used for the construction of genomic libraries of whole genomes. BAC libraries are preferred to YAC libraries for their simpler realization and higher stability. In cultivated plants, these BAC libraries are useful for positional gene cloning, genomic structural analyses, comparative analyses of genomes of related species, saturation maps with molecular markers and microsatellites extracted from specific regions. For durum wheat is now available a BAC library realized in the LANGDON cultivar, composed of 516.096 clones singularly maintained in 1344 plates with 384 clones (Cenci *et al*., 2003). This BAC library has a 5X genome coverage and supplies a high probability to identify and to isolate the desired gene. A first use has been that to isolate clones containing gene sequences coding for the phytoene sinthase, phytoene desaturase and carotene desaturase, enzymes involved in the carotenoid biosynthesis (Cenci *et al*., 2004).

Genome sequencing and associated bioinformatics resources are now a popular research tool in wheat for accelerating the analysis of genome structure and function because it leverages similar work from other crops and plants. Despite wheat is one of the world's most important crops, progress in wheat genomics has been slow due to its large and complex genome. Several studies, coordinated by the International Wheat Genome Sequencing Consortium (http://www.wheatgenome.org) are in progress with the aim of obtaining and characterizing the wheat genome. One way to reduce the genome complexity is to purify single chromosomes using flow cytometry and to perform the analysis at the sub-genomic level (Doležel *et al*., 2007). The massively parallel 454 pyrosequencing was recently used to obtain a 2x coverage of wheat chromosome 5A (Vitulo *et al*. (2011) and the resulting sequence assembly was used to identify TEs, genes and miRNAs, as well as to infer a virtual gene order based on the synteny with other grass genomes. Repetitive elements account for more than 75% of the genome, while the coding fraction represents 1.08% and 1.3% of the short and long arm, respectively, projecting the number of genes of the whole chromosome to approximately 5,000. A particularly challenging task is the anchoring of BAC contigs to a genetic map, for which the availability of high density linkage maps are crucial. In wheat, the limitations of the large genome size and lack of polymorphism can be overcome by targeted mapping, made possible by the isolation of more than 400 deletion lines for the 21 chromosomes of the wheat cultivar Chinese Spring. The deletion mapping strategy has allowed to provide deletion maps for wheat the 5A and 5B chromosomes and a genetic map of 5A enriched with popular microsatellite markers, which could be compared with other existing maps and useful for mapping major genes and QTLs (Gadaleta *et al*., 2012).

Using next-generation sequencing technologies it is possible to resequence entire plant genomes or sample entire transcriptomes more efficiently and economically. Rather than sequencing individual genomes, it is possible the sequencing of hundreds of related genomes to sample genetic diversity within and between germplasm pools. Next-generation sequencing (NGS) technologies can be applied in some important areas such as the large-scale development of molecular markers for linkage mapping, association mapping, wide crosses and alien introgression, epigenetic modifications and population genetics to advance crop genetics and breeding (see review by Varshney *et al*. 2009). The application of complexity reduction of polymorphic sequences (CRoPS ®) technology for the discovery of SNP markers in durum wheat has been reported by Trebbi *et al*. (2011). A next-generation sequencing experiment was carried out on reduced representation libraries obtained from four durum cultivars and SNP validation was carried out on a panel of 12 cultivars. A total of 2,659 SNPs were identified on 1,206 consensus sequences. Of these SNPs, 157 were mapped in one of two mapping populations (Meridiano × Claudio and Colosseo × Lloyd) and integrated into a common genetic map. The validated CRoPS-derived SNPs showed valuable features for genomics and breeding applications such as a uniform distribution across the wheat genome, a prevailing single-locus codominant nature and a high polymorphism.

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