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Changes in yield components, morphological, physiological and fruit quality traits in processing tomato cultivated in Italy since the 1930's

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ABSTRACT

Yield of processing tomato (*Solanum lycopersicum* L.) increased (~ 50%) in Italy since the 1930's. The aim of this work was to assess the changes in yield components associated with morphological, physiological and fruit quality traits in processing tomato cultivars cropped in Italy from the mid-1930s until nowadays, introduced by breeding in six representative cultivars. Marketable yield showed an increase of 0.6% *per* year of release since the 1930's. The highest marketable yield was obtained in modern cultivars due to a higher harvest index, fruit number and the ratio between ripe fruit and total fruit in comparison with the old ones. However, no single trait drove the highest marketable yield in modern cultivars. In fact, both morphological (smaller plant height and leaf area index) and physiological (accelerated plant senescence, higher leaf nitrogen status, and lower potential plant water and chlorophyll contents) traits contributed to increase marketable yield in modern cultivars. Moreover, total plant dry weight (shoot + fruit) of a single plant decreased, whereas its total fruit fresh weight and fruit dry matter content were stable and not correlated with the year of release, thus suggesting that a higher sink strength and homogeneity of fruit ripening were also involved in the highest marketable yield showed by modern cultivars. A great effort of breeders was done in the improvement of important fruit quality traits required by Italian canning industries. Fruit colour and Brix yield were positively correlated with the year of release, while viscosity and total carotenoids were negatively correlated with the year of release. However, no improvement was achieved for important traits such as soluble solids content, fruit dry weight and total fruit yield, which instead should be considered in the future breeding programmes, to improve both yield and quality of processing tomato.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important cash crops worldwide. The European tomato production in the last year was of ~ 17 million tons with an average yield of ~ 67.5 t ha^{-1}, Italy is the most important country in Europe with a production of ~ 6 million tons and an average yield of ~ 61.9 t ha^{-1} (FAOSTAT, 2018).

Once considered inedible, nowadays tomato is one of the most extensively consumed horticultural crops. Tomato can be used either directly as fresh fruit or in the form of various processed products such as paste, whole peeled, diced, juices and soups (Grandillo et al., 1999); therefore, the market is oriented to recognise two groups of cultivars, the ones for fresh market tomato, and the ones for industry.

Breeding efforts in tomato started more than 200 years ago (Foolad, 2007). Until the 1950s, tomato breeding developed multipurpose cultivars to meet several needs. Some interesting traits were introduced such as tolerances to abiotic stresses, broad adaptability to different environments and early fruit maturity. Subsequently, breeding objectives depended upon the method of production: open field *vs* greenhouse production, and whether the fruits are used fresh or processed (Stevens and Rick, 1986; Tigchelaar, 1986). In the mid 1980's, emphasis was placed on production of F1 hybrids (Grandillo et al., 1999). However, the use of hybrids in tomato is more due to protection of breeders' research investment than the benefits of heterosis *per se*

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(Georgiev, 1991; Scott and Angell, 1998). The universal goals of tomato breeding are to increase fruit yield *per* unit area and fruit quality (Bai and Lindhout, 2007; Foolad, 2007). From 1961 until nowadays the European tomato yield increased by ∼ 250% (FAOSTAT, 2018). It is estimated that on average about half of the increase in crop productivity was due to cultivar improvement through breeding (Grandillo et al., 1999).

Processing tomato needed the introduction of specific morphological and phenological traits such as: determinate growth habit, concentrated flowering and fruit set, canopy suitable for once-over machine harvest, easy separation of fruit from the vine (*jointless* characteristic) (Barrios-Masias and Jackson, 2014; Stevens and Rick, 1986). Moreover, also specific fruit quality traits are required, such as: low pH, high soluble solids, total solids and viscosity (Foolad, 2007; Gould, 1992; Hanna, 1971). In California, Barrios-Masias and Jackson (2014) compared eight processing tomato cultivars released in the past 80 years, and they reported that the modern cultivars displayed some phenological traits (early flowering and concentrated fruit set) linked to morphological ones (smaller canopy and low vegetative biomass), reporting gains in biomass nitrogen (N) concentration and photosynthetic rates.

In processing tomato, the current yield suggested that an important portion of the yield gains might also be attributed to innovative agronomic strategies. In fact, during the mid-1950s, the adoption of mechanisation and innovative agricultural practices such as new synthetic nitrogen fertilisers and plant protection products allowed an improvement of weed control and plant performance, reducing the yield losses (Barrios-Masias and Jackson, 2014; Grandillo et al., 1999; Foolad, 2007; Tester and Langridge, 2016; van der Ploeg et al., 2007). Moreover, the introduction of drip irrigation (started during the mid-1960s) favoured significant increases in yield, however, associated with a slight decrease in total soluble solids content (Grandillo et al., 1999).

Nowadays, improving fruit quality is one of the major aims of tomato breeding programs. The most relevant fruit quality traits, both in processing and in fresh market tomatoes include fruit size and shape, total solids, colour, firmness, ripening, nutritional quality and flavour (Fridman et al., 2000).

To the author’s knowledge the breeding efforts occurred in processing tomato cultivars cropped in Italy over the past decades are not completely investigated. Hence, the present study used six representative processing tomato cultivars cultivated in Italy since the 1930’s, assessing the changes in yield components associated with morphological, physiological and fruit quality changes. The final aim was to identify those traits that could be considered in the future breeding programs able to increase both fruit yield and quality.

2. Material and Methods

2.1. Plant material

Six representative cultivars of processing tomato (Table 1), cropped in Italy from the mid-1930s to 2002, were evaluated in the open field trials.

The cultivars used in these experiments (Table 1 and Fig. 1) were chosen as they were the ones most commonly cropped in Italy since the 1930’s (Ronga et al., 2018).

All the seeds were provided by Dr. P. Passeri from the ISI Sementi S.p.A. seed company, Fidenza, Italy. The six cultivars have different level of resistance/tolerance to biotic stresses, such as fungi and nematodes (Table 1).

The main characteristics of the six cultivars were: (1) “PEARSON”, released in the mid-1930s, self-topping, semi-determinate, with dense foliage and globular and large fruits, used primarily for canning; (2) “C33”, released in the early-1970s, short bush, determinate, with globular and large fruits, used both for canning and fresh market; (3) “HEINZ 2274”, the first cultivar with high fruit firmness suitable for a more efficient mechanised harvesting and durable storage, released in 1975, determinate, with medium round and dark red fruit; (4) “E6203”, released in 1984, determinate, with abundant flowering and medium blocky fruit; 5) “BRIGADE”, released in 1989, determinate, rustic and highly productive, with medium blocky fruit with good consistency; 6) “HEINZ 3402”, released in 2002, determinate, rustic, bushy with high plant vigour and fruit production, with medium oval fruit, excellent for canning (Ronga et al., 2018; P. Passeri, the ISI Sementi S.p.A. seed company, Fidenza, Italy, personal communication).

Table 1
Cultivar assessed with their year of release and disease tolerance.

Cultivar	Year of release	Ve	Fo	N	Breeder	Propagation	Origin
PEARSON	1930	*			UCD and Utah AES	OP	California
C33	1970	*			CAMPBELL	OP	Ohio
HEINZ 2274	1975	*			HEINZ	OP	California
E6203	1984	*			FERRY MORSE	OP	California
BRIGADE	1989	*	*		ASGROW	F1 hybrid	California
HEINZ 3402	2002	*	*	*	HEINZ	F1 hybrid	California

Ve = *Verticillium* spp., Fo = *Fusarium oxysporum* f.sp. *lycopersici* (1 and 2) and N = Galligen Nematoda (*Meloidogyne incognita, arenaria, javanica*). UCD = University of California, Davis; AES = Agricultural Experiment Station; OP = open-pollinated.

2.2. Growth conditions and experimental design

The open field studies were performed in the Po Valley at Fidenza, Emilia Romagna Region, Northern Italy, during the growing seasons 2013 and 2014 (Table 2).

The weather conditions were typical of the continental climate and the minimum and the maximum average temperatures, recorded over the two growing seasons, were 15.4 °C and 28.2 °C, respectively, and the rainfall during the period from transplant to harvest was 64.1 mm (Table 2).

The field site soil was an Alfisol, according to American classification of Soil Taxonomy (USDA, 2006), characterised by a clay-loam texture with a good content of total nitrogen, phosphorus, potassium and organic matter (Table 3).

Tomato seedlings, in both years, were transplanted at the end of April when they were 6 weeks old, at the fourth true leaf stage. Plant density was maintained identical, at 3.6 plants m^{-2}, for all cultivars tested in each year. Seedlings were transplanted into single rows, with a spacing of 1.40 m between each row and 0.20 m among plants in the row. The experimental design was arranged in a completely randomised design with three repetitions (thereafter called plot) for each cultivar, and planting two border rows for each plot. The plot was 5.0 m long × 4.2 m wide and contained 75 plants.

All cultivars were cropped following standard modern agronomic practices. Regarding fertilisation 166 Kg of N ha^{-1}, 194 Kg of P ha^{-1} and 258 Kg of K ha^{-1} were applied. Nitrogen was applied 33% at transplant and 67% from full flowering to fruit ripening, while P and K were applied one month before the planting time. The irrigation water was distributed by drip irrigation. The irrigation water volume was determined on the basis of the total of water lost by evapotranspiration calculated according the formula: ETc = ETo × Kc, where ETo is the reference evapotranspiration and Kc is the crop coefficient of tomato (Allen et al., 1998). When the soil was depleted to 40% of total available water, 100% ETc was restored in agreement with the evapotranspiration method of Doorenbos and Pruitt (1977). During the trials irrigation water (∼ 450 mm) was applied by drip irrigation. Weeds and pests were controlled according to the production rules of Emilia Romagna Region, Italy. In particular, weeds were controlled by chemical management (metribuzin) and using mechanical control. As regards the

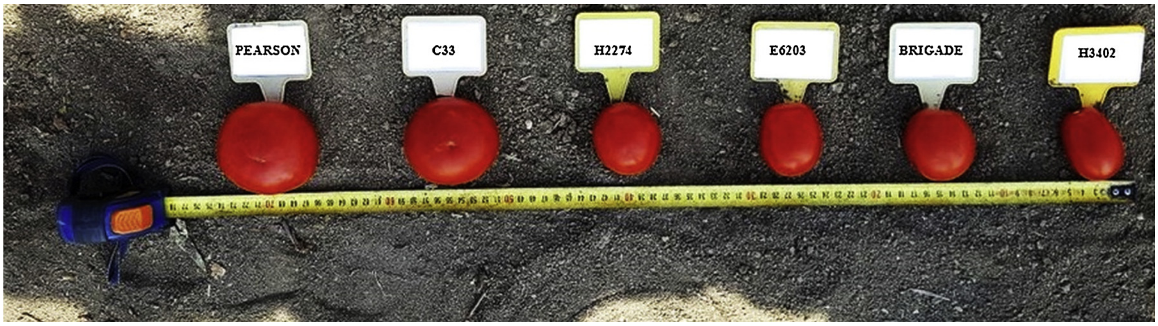

Fig. 1. Representative fruits of the six different processing tomato cultivars (left to right): Pearson (1930); C33 (1970); HEINZ 2274 (1975); E6203 (1984); BRIGADE (1989) and HEINZ 3402 (2002).

pathogen and pest control, fungicides (sulphur, copper oxychloride, difenoconazole and fosetyl-aluminium) and pesticides (azadirachtin A, imidacloprid, spinosad, abamectin and emamectin benzoate) were used. A single harvest was carried out at the end of the growing seasons, *i.e.* within the first ten days of August 2013 and 2014, when ripe fruits were at least approximately 85% of the total fruits, considering the average field value.

2.3. *Traits assessed during the crop growth*

During the two growing seasons, starting from one month after transplant, several traits were assessed in four plants *per* plot. The traits were scored at the following four stages through the crop cycle: (1) full flowering (stage 6.3); (2) beginning of fruit development (stage 7.1); (3) fruit and seed ripening (stage 8.1); (4) fruit maturity (stage 8.9) (Meier, 2001). The traits were divided into four categories (morphological, physiological and fruit quality) with a total of 29 recorded parameters. Morphological (non-destructive) and physiological traits were assessed at each crop stage, while regarding morphological (destructive) traits such as biomass and fruit quality were assessed only at harvest time.

For morphological characterisation, angle of leaf (measuring the angle, in degree, between the stem and leaf petiole of the youngest fully expanded leaf), stem (SN), leaf (LN), truss (TN) and fruit (FN, counting unripe and ripe) numbers and height of plants (H) were recorded at each sampling time. At harvest time, leaf area index (LAI) was measured using a subsample of fresh leaves that were run through the leaf area meter LI-3000A (LI-COR Inc., Nebraska, USA), and linked to dry weight of leaves (LA = area of subsample/dry weight of subsample x dry weight of sample). We referred to leaf blade and petiole as a leaf. Marketable fruit yield (considering only the fresh weight of ripe fruit), average fruit weight (considering only full ripe fruit), total fruit (as the sum of the fresh weight of unripe and ripe fruits) and the ratio between ripe fruit and total fruit were recorded. Moreover, the dry weight (DW) of different organs of the processing tomato plants as the leaves (LDW), stems (SDW) and fruits (FDW, as the sum of unripe and ripe fruits) were weighed, recorded and oven-dried at 65 °C until constant weight to obtain the dry weight of single organs and the total dry weight (TDW = LDW + SDW + FDW) and the ratio between FDW and TDW was calculated as harvest index (HI).

Regarding the physiological characterisation, the index of leaf chlorophyll content (SPAD) was estimated on the youngest fully expanded leaf by SPAD-502 (Minolta, Japan). Dualex 4 Scientific (FORCE-A, Orsay, France) was used to estimate the nitrogen balance index.

Table 3
Soil characteristics.

Soil characteristics	2013	2014
Sand (%)	8.8	9.9
Silt (%)	49.5	47.4
Clay (%)	41.7	42.7
pH	8.0	8.5
EC (dS m^{-1})	0.2	0.1
Limestone (%)	4.1	16.7
K_2O (mg/Kg)	481.0	362.8
P_2O_5 (mg/kg)	45.8	54.9
N. tot. (‰)	1.3	1.6
Organic matter (%)	1.7	1.8
CSC (meq/100 g)	20.7	23.9

Several vegetation indices were derived from canopy reflectance spectra taken with a Field Spec Hand Held VNIR spectroradiometer (ASD Inc., Boulder, CO, USA) with a wavelength range of 325–1075 nm, a wavelength accuracy of ± 1 nm and a spectral resolution of < 3 nm at 700 nm. Measurements were taken at a distance of 1 m above the canopy. Ten average spectra, each a mean of 10 spectra, were recorded *per* plot. The indices were: Normalised Difference Vegetation Index (NDVI), Plant Senescence Reflectance Index (PSRI) and Normalised Water Index (NWI) as suggested by Merzlyak et al. (1999); Prasad et al. (2007) and Rouse et al. (1974).

Finally, a sub sample of fresh fruit was used to assess the fruit quality. After pulping of ~ 35 fruits (cold break preparation), the following parameters were evaluated: pH, total soluble solid content (°Brix), viscosity, colour and total solids. The pH was measured with a Basic 20 pH–meter (Crison, Instrument, Barcelona, Spain), while °Brix were determined using a digital refractometer (HI 96814, Hanna Instruments, Villafranca Padovana, Italy). Moreover, °Brix yield was determined by multiplying the marketable yield (t ha^{-1}) by the °Brix value and dividing by 100 and is expressed as of soluble solids yield (t ha^{-1}). For viscosity, Bostwick readings were assessed for each sample as reported by Ranganna (2011). The results were recorded as distance travelled (cm) in 1 min. The experiment was performed at room temperature and repeated three times. Pulp colour was assessed using a

Table 2
Field trial sites of processing tomato for the 2013 and 2014 growing seasons.

Year	Location (Lat Long)	Transplaning date	Harvest date	Average T min (°C)	Average T max (°C)	Average seasonal rainfall (mm)
2013	Fidenza (PR) 44° 50' 48.5” N 10° 1' 50.1” E	04/30/2013	08/28/2013	15.6	28.6	66.9
2014	Fidenza (PR) 44° 50' 45.22” N 10° 1' 51.34” E	04/30/2014	08/25/2014	15.2	27.7	61.2
Average				15.4	28.2	64.1

Lat = latitude, Long = longitude, T = temperature, min = minimum, max = maximum.

Gardner XL-23 tristimulus colorimeter (Gardner Laboratory Inc., Bethesda, MD, USA), values are reported as the ratio of chromaticity indices "a" (redness) over "b" (yellowness). Total solids were determined by gravimetry (Zanoni et al., 1998). Samples were dried in a vacuum oven at 70 °C for 1 h at 600 mbar residual pressure and subsequently for 4 h at 50 mbar residual pressure. The air was dried by a Drierite™ drying column (Generalcontrol S.p.A, Milan, Italy).

For total polyphenols and carotenoid content characterisations, tomato fruits (~ 1000 g) were grossly chopped and then homogenised by an Osterizer blender (OS6805 Oster®, Di Giovanni Srl, Bologna, Italy) at low speed, under insufflation of a pure nitrogen stream to prevent oxidation.

Total polyphenols content was analysed using the Folin-Ciocâlteu method (Singleton et al., 1999). In a 10-mL flask, 0.2 ml of the clear sample (previously centrifuged), 6 ml of water, and 0.5 ml of the Folin-Ciocâlteu reagent were added. After 1 min of shaking with a vortex, 15% aqueous sodium carbonate (2 mL) was added and the solution was made up to 10 ml with water. Finally, this solution was mixed and left to stand at ambient temperature for 120 min. Absorbance was read at 700 nm (V-730 UV/Vis spectrophotometer, Jasco Europe Ltd, Cremella, LC, Italy) against a blank represented by the reagents only and compared with a standard gallic acid calibration curve (R^2 = 0.9994). Results of triplicate on different sample batche analyses are given as mg g^{-1} of gallic acid equivalents.

Total carotenoid determinations were carried out using the method described by Fish et al. (2002), with slight modifications. Briefly, 1 g of each tomato sample was weighed into a centrifuge tube and extracted until the residue was colourless (three extraction cycles) with a mixture consisting of 1 ml hexane, 0.5 ml ethanol, and 0.5 ml acetone (containing 0.05% w/v butylated hydroxytoluene). Samples were tightly sealed and shaken for 15 min. Afterwards, 0.6 ml of deionised water were added, the sample was shaken for another 5 min and left to stand to favour the solvent phase separation. The pooled hexane phases were made up to 6 ml and finally diluted 1 in 10 (total dilution factor = 60). The absorbance of the upper hexane layer was read at 450 nm and 471 nm, against a blank of pure hexane. Total carotenoids (expressed as mg 100 g fresh weight^{-1}) were calculated using the 2500 as the specific extinction coefficient E (1%, 1 cm) at the maximum absorption 450 nm (λ_{max}) (Britton, 1995).

2.4. Statistical analysis

Figures shown in the present study reported the traits assessed in each cultivar as an average of values recorded at each timing over the two growing seasons. Data of each trait was regressed against the year of release in order to determine the possible correlation between each trait and the year of release. The regression coefficients were evaluated for statistical difference from zero by F tests. Cultivar differences were investigated by ANOVA and Tukey test (at $P < 0.05$) was used to compare the means.

Moreover, a Principal Component Analysis (PCA) (Jackson, 1991; Wold et al., 1987) was performed, using for each cultivar the average values of the different replicates. Results were visualised with biplots. Since the variables analysed in the present work have different scales, before calculating the PCA models they were pre-processed using autoscaling in order to give a priori the same importance to each original variable. The number of significant PCs, *i.e.*, the number of PCs bearing useful information, indicates the number of fundamentally different properties exhibited by the data set; in the present work, two significant PCs were selected.

Statistical analysis was performed with GenStat 17th software.

3. Results

Fig. 2 shows a component hierarchy contributing to the increase in marketable yield of processing tomato. The increase in marketable yield might be ascribed to an increase in HI and a change in sink source ratio.

Single harvest of fruit was performed on 28 Aug. 2013 and 25 Aug. 2014 (Table 2). Considering all the produced fruit (unripe and ripe) by plants, the total fruit yield did not increase with the year of release of the cultivars and no differences were recorded among the cultivars (Fig. 3A).

However, considering only ripe and marketable fruits, yield increased significantly with the year of release of the cultivars (R^2 = 0.87) (Fig. 3B). The marketable yield displayed an increase of ~ 0.6% *per* year corresponding to ~ 0.48 t ha^{-1} year^{-1}, and the most modern cultivar “HEINZ 3402” recorded significantly higher commercial yield, +46%, if compared to the oldest one “Pearson”.

3.1. Morphological traits recorded during the cropping season

Fig. 4 reports eight important morphological traits: stem, leaf, truss and fruit numbers *per* plant, average fruit weight, leaf angle, plant height and the ratio between ripe fruit and total fruit that breeding putatively considered in processing tomato in the past decades (Fig. 4A-H).

Leaf number *per* plant (Fig. 4B), plant height (Fig. 4F) and average fruit weight (Fig. 4G) showed a reduction with the year of release of the cultivars (R^2 = 0.94, 0.93 and 0.92, respectively). Leaf number *per* plant decreased from ~ 52 (in the oldest cultivar) to ~ 40 (in the most modern one) (Fig. 4B). Plant height decreased from ~ 68 cm (in the oldest cultivar) to ~ 45 cm (in the most modern one), showing a reduction of ~ 0.35 cm *per* year. Average fruit weight decreased from ~ 260.0 g (in the oldest cultivar) to ~ 78.0 g (in the most modern one), showing a reduction of ~ 2.53 g *per* year. On the other hand, fruit number *per* plant (Fig. 4D) and the ratio between ripe fruit and total fruit (Fig. 4H) were the morphological traits that increased with the year of release of the cultivars (R^2 = 0.75 and 0.88, respectively). The most modern cultivar “H3402” recorded more than a 3-fold increase of the number of fruits and +42% of the ratio between ripe fruit and total fruit compared to the oldest one “Pearson”.

3.2. Morphological traits recorded at harvest

Fig. 5 shows the aboveground dry weight production of the different organs (stem, leaf, and fruit) and the total dry weight (Fig. 5A-D).

Total dry weight considered as the sum of stems, leaves and unripe and ripe fruits, was the only morphological trait that recorded a statistically significant variation, showing a reduction with the year of release of the cultivar (R^2 = 0.68). Total dry weight decreased from ~ 800 g plant^{-1} (in the oldest cultivar) to ~ 500 g plant^{-1} (in the most modern one), showing a reduction of ~ 0.5% *per* year corresponding to ~ 4.3 g plant^{-1} *per* year (Fig. 5D).

Fruit dry weight remained substantially equal through decades, on the basis of a single plant, and including both ripe and unripe fruits.

Stem and leaf dry weights recorded differences among the cultivars. Cultivars “H2274” and “Pearson” recorded higher values both of stem and leaf dry weights (+160% and +129%, and +113% and +78%, respectively) compared to the more modern “H3402”.

3.3. Leaf area index and physiological traits

Fig. 6 reports the leaf area index and the main physiological traits recorded in the present study: SPAD, NWI, PSRI, NDVI and HI that breeding changed in processing tomato in the past decades (Fig. 6A-F).

LAI and all the investigated physiological traits showed variations with the year of release of the cultivar, except for NWI. Moreover, each trait reported on the Fig. 6 highlighted interesting differences among the investigated cultivars. LAI (Fig. 6A), SPAD (Fig. 6B) and NDVI (Fig. 6E) showed a reduction (R^2 = 0.59, 0.58 and 0.61, respectively), while PSRI (Fig. 6D) and HI (Fig. 6F) reported an increase of the values (R^2 = 0.60 and 0.54, respectively) with the year of release,

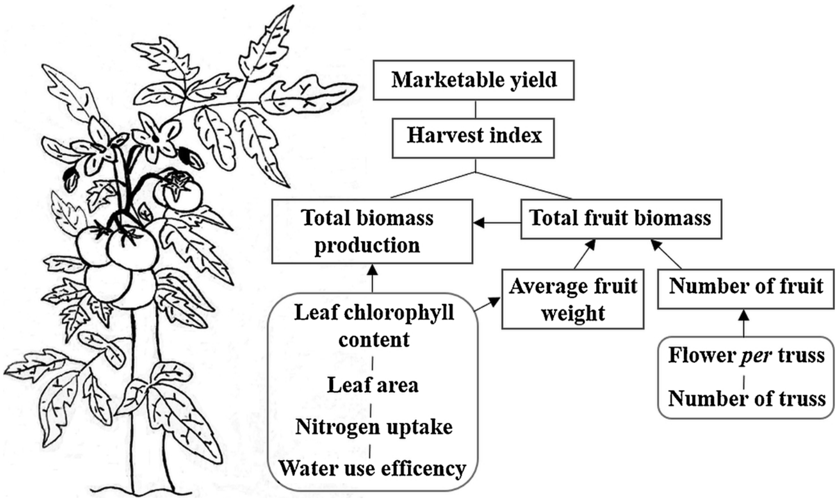

Fig. 2. Marketable yield components and yield-related traits of processing tomato. Modified from Higashide and Heuvelink.

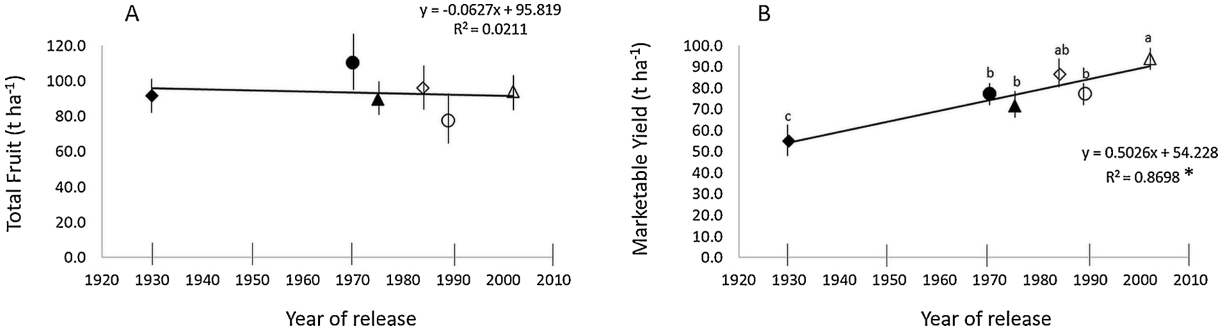

Fig. 3. Yield traits. Total fruit (A) and marketable yield (B) for six processing tomatoes released in different years: Pearson (◆), C33 (●), HEINZ 2274 (▲), E6203 (◇), BRIGADE (○), HEINZ 3402 (△). Regression line based on the data for the six cultivars. Different lowercase letters are significantly different at $P < 0.05$, according to Tukey's test. Bars represent standard error. x = year-1930. * = significant correlation at ($P < 0.05$).

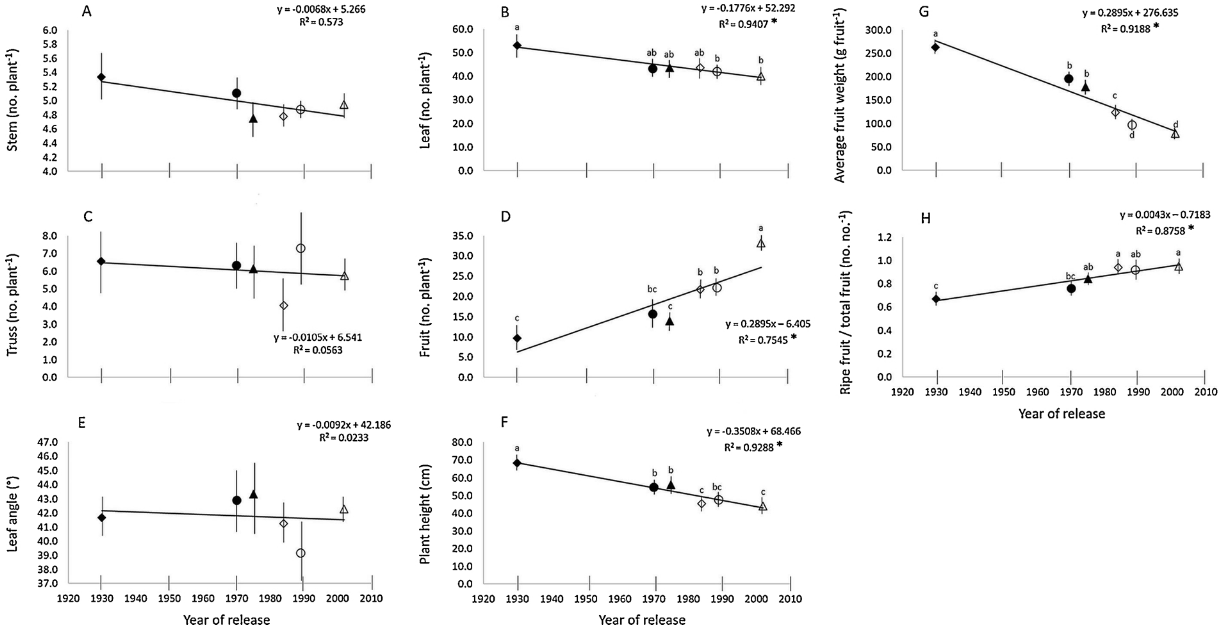

Fig. 4. Morphological traits. Stem number (A), leaf number (B), truss number (C), fruit number (D), leaf angle (E), plant height (F), average fruit weight (G) and ratio ripe fruit / total fruit (H) for six processing tomatoes released in different years: Pearson (◆), C33 (●), HEINZ 2274 (▲), E6203 (◇), BRIGADE (○), HEINZ 3402 (△). Regression line based on the data for the six cultivars. Different lowercase letters are significantly different at $P < 0.05$, according to Tukey's test. Bars represent standard error. x = year-1930. * = significant correlation at ($P < 0.05$).

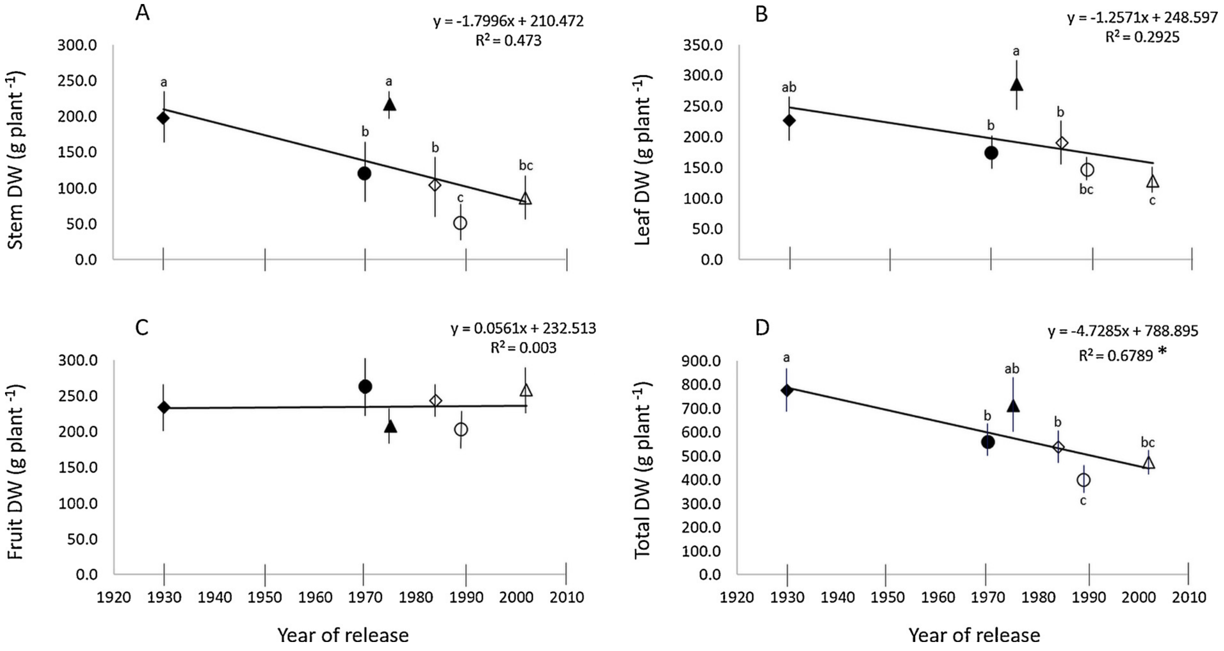

Fig. 5. Biomass traits. Stem dry weight (DW) (A), leaf DW (B), fruit DW (C) and total DW (D) for six processing tomatoes released in different years: Pearson (◆), C33 (●), HEINZ 2274 (▲), E6203 (◇), BRIGADE (○), HEINZ 3402 (△). Regression line based on the data for the six cultivars. Different lowercase letters are significantly different at $P < 0.05$, according to Tukey's test. Bars represent standard error. x = year-1930. * = significant correlation at ($P < 0.05$).

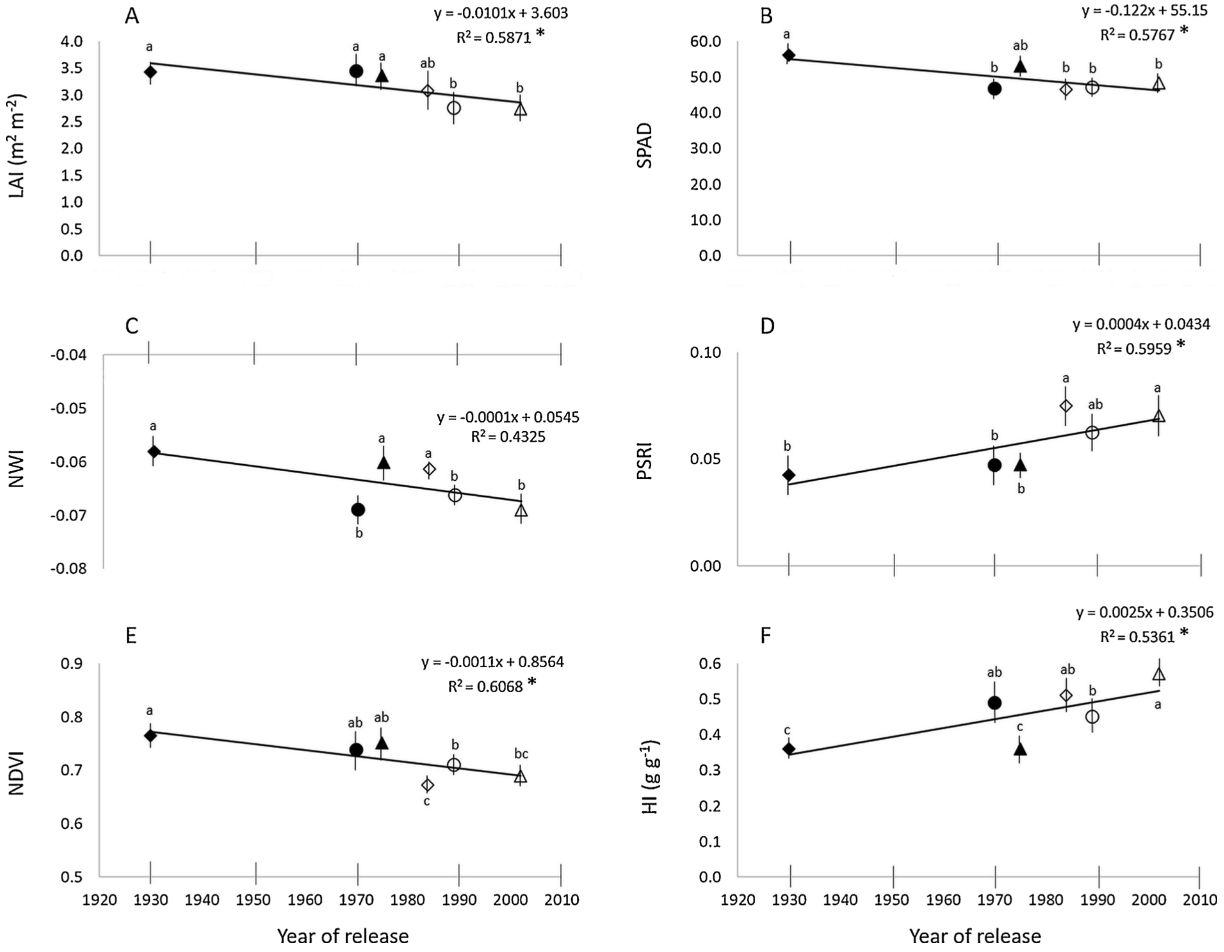

Fig. 6. Leaf area index and physiological traits. Leaf area index (LAI) (A), SPAD = index of chlorophyll content in leaf (B), normalised water index (NWI) (C), plant senesce reflectance index (PSRI) (D), normalised difference vegetation index (NDVI) (E), harvest index (HI) (F) for six processing tomatoes released in different years: Pearson (◆), C33 (●), HEINZ 2274 (▲), E6203 (◇), BRIGADE (○), HEINZ 3402 (△). Regression line based on the data for the six cultivars. Different lowercase letters are significantly different at $P < 0.05$, according to Tukey's test. Bars represent standard error. x = year-1930. * = significant correlation at ($P < 0.05$).

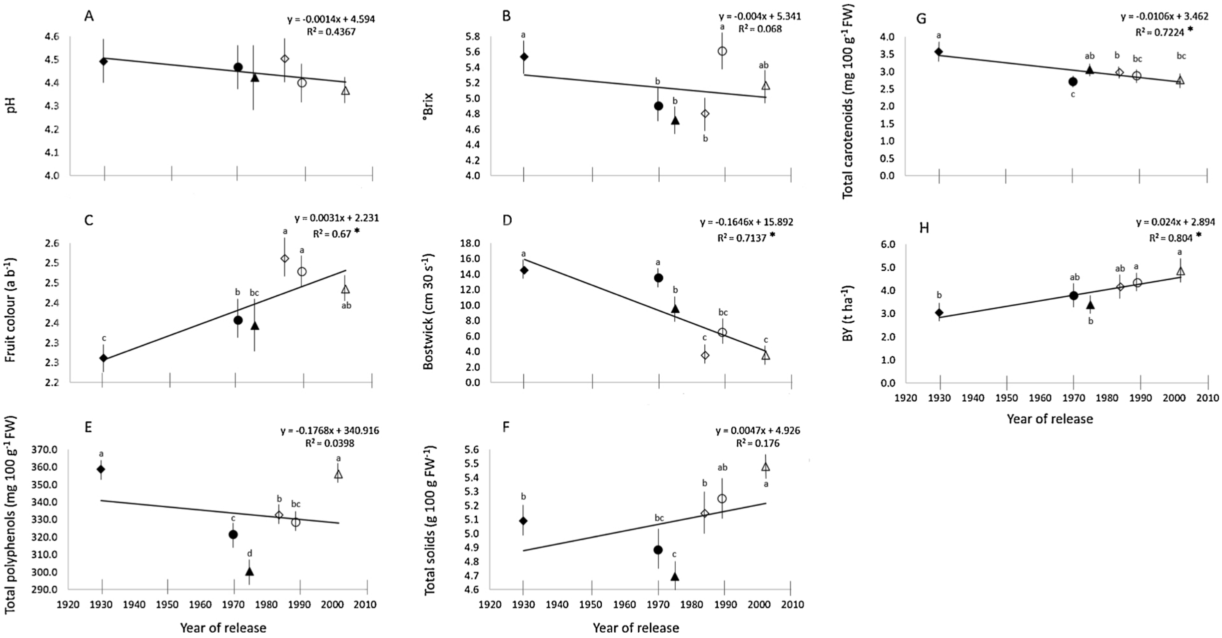

Fig. 7. Fruit quality traits. pH (A), °Brix (B), fruit colour (C), Bostwick as viscosity (D), total polyphenols (E), total solids (F), total carotenoids (G) and Brix t ha^{-1} (BY) (H) for six processing tomatoes released in different years: Pearson (◆), C33 (●), HEINZ 2274 (▲), E6203 (◇), BRIGADE (○), HEINZ 3402 (△). Regression line based on the data for the six cultivars. Different lowercase letters are significantly different at $P < 0.05$, according to Tukey's test. Bars represent standard error. x = year-1930. * = significant correlation at ($P < 0.05$).

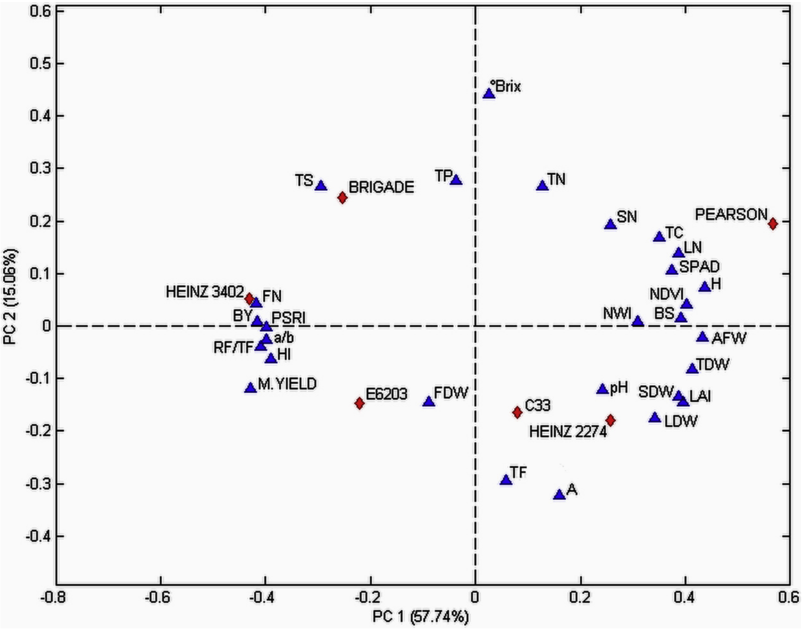

Fig. 8. Ordination biplot for the principal component analysis outputs. Red rhombus indicates the assessed cultivars, while blue triangles indicate the investigated traits. TS = total solids, TP = total polyphenols, TN = truss number, SN = stem number, TC = total carotenoids, LN = leaf number, SPAD = index of chlorophyll content in leaf, H = plant height, NDVI = normalised difference vegetation index, BS = Bostwick as viscosity, NWI = normalised water index, TDW = total dry weight, SDW = stem dry weight, LDW = leaf dry weight, LAI = leaf area index, TF = total fruit, A = leaf angle, FDW = fruit dry weight, FN = fruit number, AFW = average fruit weight, RF/TF = ratio ripe fruit/total fruit, BY = brix t ha^{-1}, PSRI = plant senescence reflectance index, a/b = fruit colour, HI = harvest index, M. YIELD = marketable yield (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

respectively. LAI decreased from ~ 3.6 (in the oldest cultivar) to ~ 2.7 (in the most modern one), showing a reduction of ~ 0.3% *per* year. SPAD decreased from ~ 55 (in the oldest cultivar) to ~ 50 (in the most modern one) and NDVI decreased from ~ 0.86 (in the oldest cultivar) to ~ 0.69 (in the most modern one). On the other hand, PSRI and HI increased from ~ 0.04 and 0.35 (in the oldest cultivar, respectively) to ~ 0.06 and 0.60 (in the most modern one). HI recorded an increase of ~ 2.0% *per* year. For NWI, "Pearson", "HEINZ 2274" and "E6203" recorded the highest values, while "C33", "Brigade" and "HEINZ 3402" showed the lowest ones.

3.4. Fruit quality traits

Fig. 7 shows the main results regarding fruit quality traits: pH, °Brix, fruit colour, Bostwick, total polyphenols, total solids, total carotenoids and °Brix yield (Fig. 7A-H).

Among them, fruit colour, viscosity (Bostwick), total carotenoids and °Brix yield showed interesting changes with the year of release. Moreover, cultivars significantly differed for all investigated traits, apart from pH and °Brix. Fruit colour (Fig. 7C) and °Brix yield (Fig. 7H) were positively correlated ($R^2 = 0.67$ and 0.80, respectively), while Bostwick (Fig. 7D) and total carotenoids (Fig. 7G) were negatively correlated ($R^2 = 0.71$ and 0.72, respectively) with the year of release, respectively. Fruit colour increased from ~ 2.2 (in the oldest cultivar) to ~ 2.5 (in the most modern one), showing an increment of ~ 0.1% *per* year (Fig. 7C). °Brix yield increased from ~ 2.9 (in the oldest cultivar) to ~ 5.0 (in the most modern one), showing an increment of ~ 0.1% *per* year (Fig. 7H). On the other hand, Bostwick and total carotenoids decreased from ~ 15.9 and 3.5 (in the oldest cultivar) to ~ 4.0 and 2.5 (in the most modern one), showing a reduction of ~ 1.0 and 0.4% *per* year, respectively (Figs. 7D and G).

For total polyphenols and total solids, even if not correlated with the year of release, "Pearson" and "HEINZ 3402", showed the highest values of total polyphenols, while "HEINZ 3402" recorded the highest value of total solids.

3.5. Principal Component Analysis

All measured traits and assessed cultivars were analysed using PCA to determine the association between traits and cultivars. Fig. 8 reports ordination biplots of the PCA. PC1 accounted for 57.74% of the variance, PC2 accounted for 15.06%, and their sum explained 72.80% of total variance.

PC1 clearly separates modern cultivars from old ones. In fact, modern cultivars are all on the negative quadrant, while old ones are all on the positive quadrant of PC1, respectively. The morphological and the fruit quality traits were the main drivers of the PC1 and PC2, respectively. In particular, among the modern cultivars "HEINZ 3402" and "E6203" were positively associated with high values of: fruit number, °Brix yield, PSRI, a/b, HI and marketable yield. "Brigade" was associated with total solids and total polyphenols. On the other hand, "Pearson" and "HEINZ 2274" were positively linked to high values of: stem number, total carotenoids, leaf number, SPAD, plant height, NDVI, Bostwick, average fruit weight, NWI, total dry weight, stem dry weight, LAI, leaf dry weight and pH. "C33" was associated with high values of total yield and angle of the leaf. Finally, fruit dry weight, °Brix and truss number, were close to the PC1 and not linked to any cultivar.

4. Discussion

Over the past decades, significant breeding efforts have been conducted in tomato, leading to modern cultivars. Useful information might be derived on the assessment of old and modern cultivars (originated in the USA, Table 1 and Fig. 1) based on the needs of the Italian and European markets. However, to the authors' knowledge, there are no published reports about changes due to plant breeding in tomato cropped in Italy, one of the most important country for tomato worldwide. Therefore, the present study aimed to close such a gap, by analysing such changes in processing tomato cultivars, cultivated in Italy since the 1930's and suitable to the Italian canning industry.

Raun et al. (2010) reported that crop productivity is dependent on the technological level and the interaction among genetic, environmental and crop management factors. Taking into account these considerations, the present study might allow an estimation of the genetic gains, since all cultivars were cropped in the same field trial and adopting the same agronomic management.

4.1. Yield changes

Focusing the attention on the most important trait like marketable yield, the results reported in this work indicated that significant ($P < 0.05$) genetic gain was achieved in processing tomato cropped in Italy since the 1930's. Marketable yield showed an average genetic gain of 0.6% *per* year of release after the mid-1930s (Fig. 3B).

Direct comparison between the different rates of genetic gains achieved in the present study with other published works is complicated because the environment, the periods over which gains were measured, the tested cultivars, the adopted agronomic management and the major breeding objectives were not always the same (Feil, 1992). Nonetheless, the genetic gains in marketable yield recorded in the present work (0.6% *per* year) falls into the range reported by Grandillo et al. (1999), who showed an average genetic gain of 1.5 and 0.4% *per* year for Californian and Israelian environments, respectively. Moreover, an increased yield, similar to that showed in the present study, was also observed in greenhouse tomato (Higashide and Heuvelink, 2009). In the present study, the highest marketable yield, realised by modern cultivars might be ascribed to three main traits such as fruit number, the ratio between ripe fruit and total fruit and HI (Figs. 4D, 4H, 6 F and 8) confirming the previously published data by Barrios-Masias and Jackson (2014), which assessed cultivars suitable for the Californian environment for the same three traits. The single fruit weight decreased through the past decades (Fig. 4G), however, breeding compensated this reduction by increasing the fruit numbers *per* plant (Fig. 4D). Scholberg et al. (2000) showed that HI of crops with high yield was about 65% of total biomass and similar results were recorded in this work by modern cultivars. Moreover, modern cultivars displayed higher percentage of fruit ripening associated with a lower proportion of green fruit at harvest time as already reported by Barrios-Masias and Jackson (2014).

For greenhouse tomato the increased yield was ascribed to both a higher dry matter production and light use efficiency (Higashide and Heuvelink, 2009). Thus, our results and previously published works indicate that breeding has modified different traits to improve yield in the determinate processing *vs* the indeterminate greenhouse tomato cultivars.

For total fruit (calculated as the sum of unripe and ripe fruit fresh weight) an unexpected result was recorded. In fact, total fruit was not correlated with the year of release (Fig. 3A). Moreover, the total fruit recorded in the oldest cultivar "Pearson" was similar to that recorded in the most modern one "HEINZ 3402". An opposite trend was reported by Higashide and Heuvelink (2009) in greenhouse tomato, confirming again that breeding had a different impact on common traits of the processing and greenhouse tomatoes in the past decades, maybe also linked to the plant growth determinism. Our result about stability of total fruit, confirmed the importance of the homogeneity and lack of gradual ripening of the fruit to increase the marketable yield. Another interesting observation is that the oldest cultivar "Pearson" recorded ~ 90 t ha^{-1} of the total fruit, using modern agronomic practices, while the same cultivar apparently showed a total fruit of ~ 50 t ha^{-1} during the 1960s (V. Sorrentino, from the Bio Agri Soc. Coop. canning industry, Eboli Italy, personal communication). This increase of total fruit in the oldest cultivar "Pearson" putatively suggests that total fruit gain was also ascribed to agronomic efforts with a gain of ~ 0.7% *per* year from 1960 until nowadays. In fact, after the mid-1950s, the adoption of mechanisation and innovative agricultural practices such as mineral fertilisers, plant protection products, drip irrigation and sub-irrigation, allowed an improvement of weed control and plant performance (Barrios-Masias and Jackson, 2014; Grandillo et al., 1999; Foolad, 2007; Tester and Langridge, 2016; van der Ploeg et al., 2007). Moreover, comparing yield displayed in the present study with the last available data reported in the literature, "Pearson", "C33", "HEINZ 2274" and "HEINZ 3402" performed as well as in California, New Zealand, Turkey and Spain environments, respectively (Barrios-Masias and Jackson, 2014; Burgmans and Bussell, 1983; Ersahin and Karaman, 2001; Gutiérrez et al., 2019). On the other hand, "E6203" and "Brigade" recorded lower values (~ 30%) compared to Californian and Israelian environments, respectively (May and Valencia, 1990; Lowengart-Aycicegi et al., 1998). Nonetheless, being marketable yield affected by several factors, more studies are needed to further dissect the results presented in this study by using different plant density levels and cropping systems, and more are expected by possible different responses to innovative biofertilizers (Ronga et al., 2019a, 2019b).

4.2. Morphological and physiological changes

Total biomass production is an important parameter in reaching optimal crop growth; however, the distribution of photosynthates among the different organs is a crucial step to obtain satisfactory yields as shown in this work (Figs. 5F and 8). In fact, modern cultivars showed a higher marketable yield and HI, stable fruit dry weight and lower total dry weight and LAI when compared to old ones. From a genetic and physiological point of view, the interpretation of this observation might be ascribed to a genetic improvement of sink strength, photosynthetic rate and translocation efficiency of tomato plants, as previously suggested by Barrios-Masias and Jackson (2014).

The two primary yield components of tomato are fruit number and fruit weight. Griffing (1990) highlighted a negative correlation between fruit number and average fruit weight in tomato, as a likely expected trade-off. The same trend was observed in the trials conducted in the present study. Average fruit weight and fruit number in modern cultivars were significantly lower and higher, respectively, than in old cultivars (Figs. 4G, 4D and 8). This suggests that the genetic gains achieved in marketable yield were derived from an increase in fruit number *per* plant, rather than in fruit size, as also reported by Grandillo et al. (1999).

Barrios-Masias and Jackson (2014) reported that modern cultivars showed a high fruit set and fruit classified as medium-size, and the same was noticed in the present study (data not shown). For future breeding programmes, increasing fruit size might be a trait to increase marketable yield. However, currently, the fruit size was following the industry needs. In fact, Italian canning industries required fruit with small and medium-size, especially for some specialty products such as peeled whole tomatoes (V. Sorrentino, from the Bio Agri Soc. Coop. canning industry, Eboli Italy, personal communication).

Modern cultivars showed increased homogeneity of fruit ripening (Fig. 4H), considered a strategic trait in the breeding of cultivars suitable for mechanised harvesting (Evans, 1993).

During fruit ripening stage, photosynthate can become a limiting factor suggesting a shorter vegetative growth stage and a reduced canopy size (Fischer, 2007) as shown in Fig. 4F in the modern cultivars. The potentially lower pool of photosynthate due to a lower LAI showed by modern cultivars might be partly compensated by an increase in N utilization efficiency (Evans and Fischer, 1999). In addition, an increase in the efficiency of the photosynthetic machinery was positively correlated with yield gain both in tomato (Barrios-Masias et al., 2013) and other crops such as wheat (Fischer et al., 1998). Nitrogen balance index, calculated as the chlorophyll/polyphenols ratio is an indicator of crop nitrogen status (Cerovic et al., 2012). Nitrogen balance index is based on a model N allocation to the primary and secondary metabolism compounds of the crop with variable C/N availability. A high nitrogen balance index, even if not correlated with the year of release, was observed in modern cultivars (data not shown), probably because the breeding efforts were performed on high input systems (well irrigated and fertilised) as suggested by Bolaños and Hsiao (1991) and Lammert et al. (2011).

Modern cultivars tended to have lower values of chlorophyll content (SPAD) and NDVI than old ones, both traits that are linked to N concentration. NDVI is an indicator that can be related to different physiological processes essential to achieve a good yield (Fortes et al., 2014). The lowest values of NDVI recorded in modern cultivars in the present work, might suggest a faster net assimilation rate during the growth cycle, as already reported by Barrios-Masias and Jackson (2014). However, further studies are needed to validate this hypothesis, by dissecting the photosynthetic rate through the growing cycle, also for the cultivars object of this study and in the Italian environment.

Plant Senescence Reflectance Index (PSRI) is a physiological trait related to the senescence of the canopy (Peñuelas et al., 1994). Hatfield et al. (2010) found that PSRI was sensitive to changes in leaf area during the senescence phase and this was confirmed in Fig. 8 where PSRI and LAI were negatively associated. PSRI was also positively correlated with the year of release (Fig. 6D) suggesting the increased earliness of modern cultivars assessed in the present work as also reported by Barrios-Masias and Jackson (2014).

Environmental conditions, agronomic practices, soil properties and crop growth might influence the crop water status. Water use efficiency (WUE) calculated as the ratio between yield and applied water increased ~ 50% since the 1970's (Hanson and May, 2006). In the present study WUE was not calculated; however, the modern cultivars were higher yielding than old ones (managed by applying the same amount of irrigation water) showing high PSRI and low NWI values. Modern cultivars may use as much water as old ones, but at different rates as affected by the duration of the growth period, leaf stomatal conductance and LAI, which can be translated into similar evapotranspiration but showing high yields and improved WUE (Barrios-Masias and Jackson, 2014). Normalised Water Index allows an estimation of the water status of the crop (Hanks, 1988). The plant water status is an important parameter as it gives information that can be used to select tolerant cultivar to drought stress in breeding (Köksal et al., 2008; Munjal and Dhanda, 2005). In fact, low values of NWI consistently provided the best relationships with grain yield (Prasad et al., 2007) and in agreement also with what observed in the present work (Figs. 6C and 8).

4.3. Fruit quality changes

Among fruit quality traits, °Brix is one of the most important, that is generally negatively related to yield as reported by many authors (Fulton et al., 1997; Grandillo et al., 1999; Tanksley et al., 1996). No significant genetic gain was found in °Brix for the investigated cultivars in the present work (Fig. 7B), and the same result was shown for the Californian environment, whereas in Israel an average increase of 0.53% *per* year was recorded (Grandillo et al., 1999). Another interesting trait is the Brix yield (BY), that is a trait derived from °Brix and marketable yield. The overall genetic gain was 0.9% *per* year (Fig. 7H), and a similar trend was displayed in Israel, while a gain of 1.5% *per* year was recorded in California (Grandillo et al., 1999). These differences were due to the different gains of marketable yield and °Brix as previously showed. Moreover, in Italy as well as in California, the absence of genetic gain for °Brix was balanced by marketable yield gains. On the other hand, in Israel, all the efforts were made to improve fruit quality traits rather than increase the marketable yield (Grandillo et al., 1999).

Two other important traits such as colour and Bostwick viscosity recorded significant genetic gains in modern cultivars, and the same behaviour was displayed in Israel (Grandillo et al., 1999). The gains in fruit colour might be ascribed to an increased content of lycopene. This hypothesis could be confirmed by the negative correlation found between total carotenoids and the year of release (Fig. 7G). However, further studies are needed to corroborate this hypothesis.

Most breeders focused on yield, biotic and abiotic stresses and firmness, which are essential both for long shipping and long shelf life, rather than fruit flavour quality. In fact, an interesting study demonstrated that modern cultivars contain lower fruit flavour than the old ones due to a dilution of many flavour volatiles (Tieman et al., 2017).

4.4. Future breeding efforts in processing tomato

The results of multivariate analysis, together with the evidences regarding the marketable yield and fruit quality (pH, Bostwick viscosity, °Brix yield) suggest a greater involvement of the Italian canning industry in breeding objectives since the 1930's. However, lower efforts seemed devoted to °Brix, fruit dry weight, total polyphenols, total fruit fresh weight and leaf angle, all traits that might be considered in the future breeding both to increase the marketable yield and fruit quality in processing tomato. Similar results were also reported by Liabeuf and Francis (2017), which suggested that fruit quality had less of an influence on cultivar success than yield.

Knowing the available extent of genetic diversity within cultivars is useful for successful breeding programs (Milc et al., 2016). Recently, molecular genetics and genomics developed interesting tools to overcome yield and quality limitations (Capel et al., 2017; Vargas-Ortiz et al., 2018), giving useful information for the introgression of favourable traits in elite germplasm from less adapted materials carrying useful traits (Fernandez-Moreno et al., 2017).

Finally, new breeding objectives should consider the concept of agricultural sustainability, maintaining high yield and fruit quality also under low-input farming practices and especially under adverse climate conditions; in this context crop modelling can give useful information (Cammarano et al., 2019a; 2019b; Lovelli et al., 2017; Ronga et al., 2015, 2017, 2019c, 2019d, Ventrella et al., 2012). Ronga et al. (2018) investigated the physiological responses to chilling in the same cultivars assessed in the present study, showing a better regrowth capacity in the modern cultivars compared the old ones and a similar result was also reported by Caradonia et al. (2019). Future breeding gains will be achieved only by a coordinated effort between breeders, physiologists, agronomist and ecologists, which can identify suitable traits for developing new cultivars able to counteract future issues.

5. Conclusions

Results obtained in the present study indicate that breeding efforts contributed to a significant increase of both marketable yield and fruit quality of processing tomato cultivars released in Italy since the 1930’s. In particular, HI, the ratio between ripe fruit and total fruit and fruit number were the main traits involved in the achieved marketable yield gains. Moreover, traits selected for mechanised harvesting such as smaller canopies, concentrated fruit set and homogeneity of fruit ripening likely contributed to marketable yield gains by a shorter vegetative stage and an early leaf senescence. Important fruit quality traits such as °Brix yield, colour and Bostwick viscosity recorded significant genetic gains in modern cultivars. On the other hand, °Brix, fruit dry weight, total polyphenols, total fruit fresh weight and leaf angle, were traits that remained stable in the past decades. Hence, these traits might be considered in the future breeding programmes to improve fruit yield and quality, in elite cultivars suitable both for new precision agronomic strategies and facing the environmental uncertainties of climate change.

Declaration of Competing Interest

Declarations of interest: none.

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