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# **Apple Breeding in Tunisia and the Actual Climatic Context: Quality Assessment and Crop Adaptation**

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#### **Abstract**

An apple breeding program has recently led to thirteen new apple accessions (H1 to H13) growing at the INRAT experimental station in north-east Tunisia. Assessment of Quality performances on the basis of morpho-chemical traitsshowed a large diversity between apple genotypes. Fruit size and yield were obviously different. Average fruit weight varied between 73 g (H1 samples) and 183 g (H9 fruits). A large diversity was observed in fruit shape and colour, the fruit can be globose conical (H1, H4, H6 and H12), flat (H8, H9, H10 and H11), oblate (H3, H7 and H13) or conical (H5). Ground colour of the skin varied from yellow green (H1 and H3), red green (H9, H10 and H13), yellow (H5 and H6) to red (H2, H8 and H12) and chromaticity values were strong related to external fruit colour. Fruit firmness ranged between 0.496 and 0.644 kg/cm². Total soluble solids (TSS) ranged between 13.2% and 16.5% and titratable acidity (TA) of the juice between 0.005 and 0.011 g malic acid/L. The index of maturity (IM = TSS/TA) was the highest for H2 while H7 apples exhibited the lowest IM score. Juice yield, an important criterion in postharvest technology, varied from 27% in H7 apples to 44% in H12 fruits. The results give a preliminary classification of the 13 new apple accessions using quality rating of the fruit. The range of apple strains H2, H8, H9 and H13 presented the most attractive fruit considering physical aspects, while H2, H3, H7 and H10remain interesting on the basis of flavour and quality taste of the fruit which could be somewhat explained by the advancedstage of maturity reached by these fruits at the harvest time.

#### **Keywords**

Malus Domestica, Quality Rating, Fruit Shape, Skin Colour, Maturity Index, Principal Component Analysis, Hierarchical Cluster Analysis

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# 1. Introduction

Enhanced fruit quality is one of the main objectives across the range of apple breeding programs. Fruit quality is frequently associated with pest and disease resistance of particular cultivars (Stoeckliet al., 2011). Enhanced fruit quality, by which is meant improving the combined typological traits of fruit appearance like colour, shape, size, those intrinsic to the fruit itself like taste, firmness, crispiness, juiciness, flavour, and postharvest potential like shelf-life and storability. The ability to quantify formerly qualitatively

evaluated fruit parameters should be of interest to breeders and growers. Once obtained, this information could be used to assist in cultivar development programs (Eigenmann & Kellerhals, 2007).

Apple cultivation occupies a prominent place in Tunisia. However, its production remains low and never reached the desired performance compared to northern countries (Harbi Ben Slimane *et al.*, 2013). This is mainly due to the inadequacy of cultivated varieties to local climatic conditions. An apple breeding program was started in 1960 at the INRAT (National Institute for Agricultural Research, Tunisia) with

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the aim of creating varieties adapted to local climatic conditions, in particular with low chilling needs. Three varieties ('Zina', 'Aziza' and 'Chahla') were selected in 1981 with good adaptation to mild winters and satisfactory yield but have a notable weakness to handling and poor storage performances at the postharvest (Anonymous, 1981). A new breeding program started in 1985 over collaboration with INRA Angers (France) where a series of crosses were made. The current program led recently to 13 new apple genotypes(Tradet al., 2014). The thirteen hybrids (H1 to H13) have been selected and installed in a plot in 'Mornag'. Assessment of the genetic diversity of these hybrids was made on the basis of both quantitative and qualitative traits related to the fruit. The principal objective was to address the rising bio-climate challenges and to promote adaptability and sustainability of national apple production, thus, to enhance the quality performance of local apple cultivars growing in the mild climate areas of the country. Target Parameters to consider are: quality of the fruit, low chilling needs and postharvest storability. The first term of this action was unveiled in this paper. The two other parameters will be monitored later during the next growing season.

# 2. Materials and Methods

Apples were harvested from 4-year-old trees grafted onto MM106 rootstock planted in an experimental collection plot in the INRAT experimental station of 'Mornag' in 2014. The weather conditions were usual in this North-East Tunisian location with high summer temperatures (> 35 °C) and low rainfall (304 mm). Average min and max temperatures are respectively 19 and 31°C during growing season with more than 300 hours of insolation or the warmermonths (July and august). These figures showed a slight increase compared to data from the end of the 1990sinspecting the same region with min and max temperature values of 17 and 28 °C respectively. The tree rows have a north-south orientation spaced at  $5 \times 4$  m. Apple trees were trained as an open vase system and drip irrigated. No specific crop protection practice was applied during this first cropping season. More details about the pedigree of the 13 apple genotypes are given in Table 1.

Ten fruits per tree and 5 trees per strain were harvested in mid-august 2014 from which 20 fruit were randomly picked for quality assessment of the fruit. Main descriptors for apple fruit were assessed for each strain (IBPGR, 1982; UPOV, 2005). Parameters measured were: fruit size, fruit shape, fresh weight, ground colour and russet amount expressed as the usual percentage of fruit surface russeted. Firmness was measured on both opposite fruit sides using a table penetrometer (Fruit Texture Analyser, GÜSS Manufacturing,

South Africa) with an 11 mm diameter plunger tip. Total soluble solids (TSS) were determined with a digital refractometer (OPTECH GmbH, Mûnchen, Germany) and expressed in percent (%) at 20 °C. Titratable acidity (TA), expressed as mg of malic acid/L, was determined by titrating apple juice with 0.1 M NaOH. Apple skin colour was measured with a Konica-Minolta Chromameter CR-400 portable tristimulus colorimeter (Minolta Corp, Osaka, Japan) and recorded in CommisionInternationaled'Eclairage (CIE) colour space coordinates  $(L^*, a^*)$  and  $b^*$ . The colorimeter was standardized by using the Minolta white calibration plate CR-A43. Skin colour was measured for 20 marked fruits per strain at two equatorial locations 180° apart on each fruit, on both blushed side and shaded side. In the CIE L\*a\*b\* uniform colour space, the colour coordinates are: L\* the lightness coordinate; a\*\_the red/green coordinate, with +a\* indicating red, and -a\* indicating green; and b\* the yellow/blue coordinate, with +b\* indicating yellow, and -b\* indicating blue. The L\*, a\* and b\* coordinate axis defines the three dimensional CIE colour space. Thus, if the L\*, a\* and b\* coordinates are known, then the colour is not only described, but also located in a quadrant (Ayala-silvaet al.,

Morphological as well chemical measurements of apple samples were collected in triplicate (n=3)with 10 fruits per replicate for morphological parameters and 20 fruits subdivided into 3 lots for chemical analysis. Data were subject to one-way analysis of variance (ANOVA) and the results were given as mean  $\pm$  standard deviation (SD). Genotypes from homogeneous subsets were displayed. Principal component analysis (PCA) was run for apple cultivars discrimination. Single linkage dendrogram showing the distance between genotypes was carried out referring to hierarchical cluster analysis. All statistics were performed using Statistical Package for the Social Sciences (SPSS version 13.0; SPSS Inc.).

**Table 1.** General description of the 13 apple genotypes (H1: hybrid 1)

No	Genotypes	Pedigree
H1	M P206bisR12V4	Golden Delicious ×Zina
H2	M P206bisR11V1	Golden Delicious ×V1(Golden ×Ajmi)
Н3	M P206bisR17A2	Jonathan ×AjmiD4Sdlg
H4	M P206bisR3V2	Golden Delicious ×V21(Golden ×Ajmi)
H5	M P206bisR4V1	Jonathan ×AjmiC29Sdlg
Н6	M P206bisR9V1	Semis de Golden Delicious
H7	M P206bisR3A14	Redspur×Jerseymac
H8	M P206bisR3A15	Redspur×Jerseymac
Н9	M P206bisR3A16	Redspur×Jerseymac
H10	M P206bisR4A8	Redspur×Jerseymac
H11	M P206bisR1A24	Redspur×Jerseymac
H12	M P206bisR1A26	Redspur×Jerseymac
H13	M P206bisR2A27	Redspur×Jerseymac

# 3. Results and Discussion

#### 3.1. Fruit Size and Yield

Total yield obtained for the different hybrids was not the same and varied between 3 kg/tree (H1) and 60 kg/tree (H4) (Table 2). Average yield value was 17 kg/tree and this could be considered as a simple estimation of apple production during this first early pick. Values for H4, H12, H11, H2 and H5 were greater than those for the other strains.

A large variability was observed in fruit shape, the fruit can be globose conical (H1, H4, H6 and H12), flat (H8, H9, H10 and H11), oblate (H3, H7 and H13) or conical (H5). Fruit shape has generally little influence on consumer preferences. Average fresh weight for the 13 hybrids ranged between 73 g and 183 g. H9 trees showed the heaviest fruit but the mean

yield value per tree was low compared to the average of all apple strains. Fruit size varied from 54 mm to 80 mm with H9 fruit bearing larger fruit compared to other genotypes. Differences in fruit size and weight between apple hybrids were highly significant ( $P \le 0.01$ ) and H9 hybrid could be subdivided in one homogeneous subset on the basis of size and fruit weight (Table 2). Similar results on variability of apple fruit weights were published by Iglesias et al. (2008) for eight 'Gala' strains with the lowest value recorded was for 'Obrogala' (171 g) and the highest value for 'Buckeye' apples (216 g). Average fresh weight for 'Mondial Gala' apples at commercial harvest in the 2000, 2001, 2002 and 2003 seasons was respectively 198 g, 141 g, 154 g and 152 g (Iglesias &Allegre, 2006). In our study H4, H9 andH10 produced the heaviest fruits with more than 150 g.

**Table 2.** Morphological description of the 13 apple genotypes (data values are means  $\pm$  standard deviations; n: number of replicates = 3)

Genotype	Yield (Kg/tree)	Fruit weight (g)	Fruit size (mm)	Firmness (Kg/cm <sup>2</sup> )	Russeting (%)
H1	3	73 ±11g	54 ±3f	0.63 ±0.03abc	25
H2	22	101 ±9ef	63 ±2de	$0.59 \pm 0.01$ cd	25
Н3	6	85 ±8fg	61 ±3e	$0.58 \pm 0.02$ de	12
H4	60	151 ±23b	69 ±3c	$0.62 \pm 0.02$ abc	12
H5	20	84 ±12fg	55 ±2f	$0.60 \pm 0.02$ bcd	12
H6	8	117 ±7de	64 ±2de	$0.50 \pm 0.01 f$	12
H7	10	132 ±2bcd	68 ±2c	$0.64 \pm 0.01ab$	0
H8	8	125 ±11cd	68 ±1c	$0.56 \pm 0.01e$	12
Н9	7	183 ±19a	80 ±3a	$0.58 \pm 0.00$ de	0
H10	16	152 ±12b	74 ±3b	$0.59 \pm 0.01$ cd	0
H11	23	114 ±4de	67 ±1cd	$0.64 \pm 0.02a$	0
H12	26	133 ±6bcd	66 ±0cd	$0.59 \pm 0.01$ cd	12
H13	13	144 ±12bc	69 ±2c	$0.60 \pm 0.01$ cd	0
MIN	3	73	54	0.50	0
MAX	60	183	80	0.64	25

Means are compared horizontally for each parameter. Identical letters (a, b, c...) refer to genotypes from homogeneous subsets performed by Duncan's multiple range test ( $P \le 0.05$ ). MIN: minimum value, MAX: maximum value, H1: hybrid 1.

#### 3.2. Firmness and Fruit Colour

Fruit firmness ranged between 0.496 and 0.644 kg/cm<sup>2</sup>. H1, H4, H7 and H11 had firmer fruits than the other apple strains. H6 fruits showed relatively the low firmness value (Table 2). Firmness is an important parameter in apple quality assessment as it can inform about storage capacity and resistance to manipulation during postharvest life. Fruit softening is often used as a criterion for estimating the feasibility of their storage or shelf life (Kader, 1992; Blankenship et al., 1997). Firmer fruit can be stored longer, however, if too pronounced firmness leads to a harder chew that can alienate a large segment of elderly consumers (Sansaviniet al., 2004). There is consensus that firmness is a good predictor of consumer preferences. The number of consumers that reject apples rapidly declines as the firmness increases to about 0.914 kg/cm<sup>2</sup> (Harker, 2001). Russeting is another important parameter in apple rating. Russeting observed on apples distorts physical appearance and is a

major cause of fruit downgrade. Russeting is a physiological disorder with no effect on taste and flavour of ripe fruits (Eccher, 1986). Russet amount was absent in H7, H9, H10, H11 and H13 fruits while it covers until 25% of the fruit surface in H1 and H2 apples.

Variability was also observed in ground colour of the skin varying from yellow green (H1 and H3), yellow (H5 and H6), red green (H9, H10 and H13) to red (H2, H8 and H12). Chromameter measurements on the skin of apple genotypes have shown large differences in L\*, a\*, b\* values ( $P \le 0.01$ ) and differences in shade of red are apparent to the eye. The darkest skinned genotypes (H2, H7, H8 and H10) had the highest a\* value and as shown in Table 4, fruits from the genotype H8 could be subdivided in one homogeneous subset ( $P \le 0.01$ ) characterized by an intense red colour of the skin. This was also true for a\*/b\* value which refer to the high colour of the fruit. The ratio a\*/b\* was demonstrated to be directly related with anthocyanin content in the fruit (Iglesias et al., 2008). In our study, H8 apples with pronounced red

colour, had the highest a\*/b\* value (Table 4). Lightness values (L\*) ranged from 53.7 for H8 to 79.4 for H5 hybrid. H5 produced yellow apples resembling to 'Golden delicious' fruits. Colour is considered to be one of the most important external factors of fruit quality as the appearance of the fruit greatly influences consumers (Singh & Reddy, 2006). With red-skinned cultivars such as 'McIntosh' and 'Delicious', red colour is very important in determining the grade of the fruit. The consumer associates red colour with good quality (Dayton, 1963). Red apples are attractive and the fresh fruit market preference in Tunisia is generally given to the better

coloured fruit. Red colour indicates maturity and full flavour and lack of colour does not adversely affect apples for processing (Dayton, 1963).

The recent cultivars growing in mild climate region of northeast Tunisia developed full coloured fruits with strong red colour for reddish skin apples and strong yellow colour for yellowish apple strains. Some apple genotypes have even developed a bright red colour on a yellow background giving to the fruit a peculiar attractiveness (Hybrid H7).

**Table 3.** Chemical and technological description of the 13 apple genotypes (data values are means  $\pm$  standard deviations; n: number of replicates = 3)

Genotype	Juice yield (%)	TSS (%)	TA (mg malic ac./L)	pН
H1	39.7 ±2.1bc	16.3 ±0.2a	8.3 ±1.1b	3.12 ±0.02efg
H2	$37.7 \pm 2.9 \text{bcd}$	16.5 ±0.1a	5.0 ±0.0d	$3.54 \pm 0.14b$
Н3	$41.0 \pm 0.0ab$	$13.9 \pm 0.7 de$	$5.0 \pm 0.0 d$	$3.16 \pm 0.12 efg$
H4	$36.0 \pm 2.6$ cd	15.3 ±0.8abcd	7.7 ±1.1bc	$3.09 \pm 0.06$ efgh
H5	$35.0 \pm 1.7d$	14.1 ±1.1de	5.0 ±0.0d	3.48 ±0.06bc
Н6	$36.0 \pm 1.0$ cd	14.3 ±0.4cde	5.7 ±0.6d	$3.23 \pm 0.18 def$
H7	$26.7 \pm 3.8e$	$13.9 \pm 0.7 de$	11.3 ±0.6a	$3.00 \pm 0.11$ gh
Н8	$35.7 \pm 2.1d$	$14.9 \pm 0.4 bcd$	5.7 ±0.6d	$3.80 \pm 0.08a$
Н9	38.0 ±1.0bcd	$14.8 \pm 0.3 \text{bcd}$	$6.0 \pm 1.0$ cd	$3.29 \pm 0.07$ cde
H10	$40.0 \pm 1.0$ ab	$16.0 \pm 0.2ab$	4.7 ±2.1d	$3.15 \pm 0.07 efg$
H11	41.3 ±2.5ab	13.2 ±1.3e	5.7 ±1.1d	$3.02 \pm 0.10 \text{fgh}$
H12	43.7 ±2.1a	14.0 ±1.2de	7.7 ±0.6bc	2.90 ±0.11h
H13	35.3 ±1.5d	15.5 ±0.7abc	7.7 ±0.6bc	$3.41 \pm 0.21 bcd$
MIN	26.7	13.2	4.7	2.90
MAX	43.7	16.5	11.3	3.80

Means are compared horizontally for each parameter. Identical letters (a, b, c...) refer to genotypes from homogeneous subsets performed by Duncan's multiple range test ( $P \le 0.05$ ). MIN: minimum value, MAX: maximum value, H1: hybrid 1, TSS: total soluble solids, TA: titratable acidity.

**Table 4.** Ground colour and chromaticity values (L\*, a\*, b\*, a\*/b\*) of the thirteen apple genotypes (H1: hybrid 1).

Genotype	Ground colour <sup>1</sup>	L*	a*	b*	a*/b*
H1	Green-yellow	65.4c	-12.3g	48.2a	-0.25fg
H2	Red	53.8e	19.2ab	26.5g	0.92ab
Н3	Green-yellow	73.3b	-13.1g	42.3b	-0.30g
H4	Green-red	65.8c	-0.9ef	38.6cd	0.01ef
H5	Yellow	79.4a	-4.8ef	36.3de	-0.13efg
Н6	Yellow	76.9ab	-6.9fg	39.6bc	-0.17fg
H7	Red-yellow	64.2c	13.0bc	32.4f	0.59cd
H8	Red	53.7e	22.8a	26.1g	1.11a
Н9	Red-green	59.3d	9.7c	28.6g	0.61cd
H10	Red-green	57.1de	14.1bc	27.3g	0.81bc
H11	Green-red	65.7c	1.6de	34.4ef	0.11e
H12	Red	59.3d	9.8c	32.8f	0.46d
H13	Red-green	59.9d	8.2cd	34.7ef	0.38d

<sup>&</sup>lt;sup>1</sup>Descriptor list for apple (IBPGR, 1982); Apple (Fruit varieties) (UPOV, 2005).

Means are compared horizontally for each parameter. Identical letters (a, b, c...) refer to genotypes from homogeneous subsets performed by Duncan's multiple range test ( $P \le 0.05$ ).

### 3.3. Soluble Solids and Titratable Acidity

Total soluble solids (TSS) and titratable acidity (TA) of the juice were different between apple genotypes and differences were highly significant ( $P \le 0.01$ ). H1, H2, H4, H10 and H13 produced rich fruit on soluble solids with respectively 16.3%, 16.5%, 15.3%, 16.0% and 15.5%. H11 fruits showed the lowest values with only 13.2%. Nevertheless, it remains superior to the minimum value established in the EU for the

commercialization of 'Gala' apples (11%) (Iglesias *et al.*, 2008). Published recommendations on minimum TSS for apples are between 12% and 14% (Harker, 2001). Sweetness is an important indicator of fruit quality and is highly correlated with ripeness in most fruit (Trad*et al.*, 2012). TSS and TA are important predictors of consumer acceptability (Harker, 2001). Titratable acidity was around 6.7 mg malic acid/L. The highest score was recorded in H7 apples with 11.3 mg malic acid/L. As a result, maturity index (TSS:TA

ratio) was higher for H2 and H10 apples. With TA =11.3 mg/L and pH =3.0, H7 cultivar yielded the highest acid fruit. Sensory studies have shown a close relationship between TA and acid taste in apples. The relationship between TA and consumer acceptability is cultivar specific (Harker, 2001). Maturity index is considered a good indicator of ripening level of the fruit when picked. Sensory characteristics largely depend on the level of ripeness reached by the fruit at harvest time (Watadaet al., 1980). For the same harvest date, H2 and H10 apples reached higher level of maturity than fruit from other strains. The sugar/acid ratio is one of the most important factors in fruit taste (Karaçali, 2002) and as studied in tomatoes (Choi et al., 1995), peaches and nectarines (Luchsinger&Walch, 1993), there is a net relationship between colour and level of maturation in these fruits. This was true for our case of study in apples. The most coloured fruits were those with the highest maturity index. Juice yield, an important criterion in apple juice manufacturing, showed a large discrepancy between the thirteen genotypes ( $P \le 0.01$ ). H12 apples produced almost 44% of juice (Table 3). With juiciness exceeding 40% (Table 2), H12, H3, H10 and H11

hybrids could be interesting in fruit juice industry. H7 apples showed the lowest value with only 27% of total fruit weight. Apple juiciness is among desirable fruit traits for evaluation of apple cultivars (Eigenmann&Kellerhals, 2007).

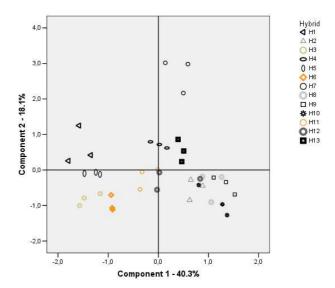


Figure 1. PCA scatterplot of 13 apple genotypes based on morpho-chemical traits of the fruit

Dendrogram using Single Linkage

#### Rescaled Distance Cluster Combine

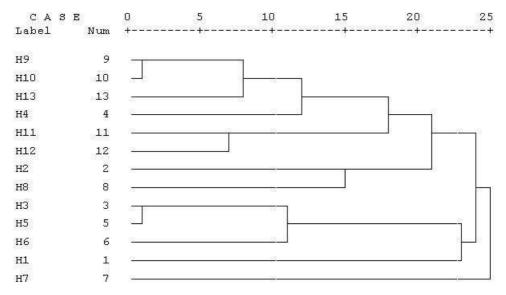


Figure 2. Dendrogram showing the relationship between the 13 apple genotypes built from morphological and chemical parameters of the fruit. (H1: hybrid 1).

#### 3.4. Principal Component Analysis

PCA performed on the basis of fruit quality attributes showed three main components explaining together 72% of the total variance. PC1 explained 40% of the variance and was correlated with a\* colour coordinate, fruit size and fruit weight (Figure 1). Fruits from H2, H8, H9 and H10 are well associated with the first component. The four apple genotypes are distinguished by a dark red skin colour and

heavy weight apples consideredimportant parameters in fresh apple marketing. PC2 accounted for 18% of the variability. PC2 was correlated with firmness and titratable acidity. Apples from H7cultivar are closely associated with the second component (Figure 1). H7 genotype is characterized by a firm and acid taste fruit and could be discriminated from the other hybrids by these two parameters. Principal component analysis separated two groups of apple cultivars on the basis of quality traits. The first group which explained

more variability existing between apple accessions highlighted H2, H8, H9 and H10 hybrids. The second group highlighted H7 apples by firmness and acidity of the juice. In figs (*Ficuscarica*), shape, size and external colour associated with sugar content represent the most interesting parameters for fruit quality assessment (Tradet al., 2012). Blazek &Paprstein (2014) have classified apple, based on the consumer preference testing in last 34 years, into two classes with taste as first preference followed by fruit appearance.

#### 3.5. Hierarchical Cluster Analysis

Dendrogram displayed in figure 2 summarizes the information collected from cluster proximity matrix. The gap comprised between 20 and 25, splits apple genotypes into two clusters. The first one includes H7 genotype. The second one is composed by the other apple strains from which however recede H1, H3, H5 and H6 hybrids. This topology confirms the divergence of H7 and with a lesser degree H1, H3, H5 and H6 with the other apple cultivars. The most closely related accessions were H9 and H10 (distance = 5.98). These two genotypes yield apples with similar quality traits and as demonstrated in table 1, derive from the same genotypic parents (Redspur×Jerseymac). H3 and H5 hybrids were also so close regarding quality of their fruit and are derived from similar individual crossing (Jonathan×Ajmi). The most distant apple genotypes were H1 and H7 (distance = 16.29). In general, apples with comparable external colour were grouped together (H4, H9, H10 and H13). Fruit size was also decisive in apple genotype classification (H11 and H12). With reference to statistical interpretations, selection of new apple cultivars for extension and field dissemination should consider but not be limited to physical aspect of the fruit. Colour and fruit size explain more the variability between apple genotypes. A first selection should be made on the basis of these two parameters. Further considerations should be given to flavour and nutritional aspects of the fruit to have a convincing judgement about quality performances of the 13 novel accessions.

## 4. Conclusions

The range of apple strains H2, H8, H9 and H13 presented the most attractive fruit considering physical aspects, while H2, H3 and H10 yielded apples with the best flavourcompared to other accessions. H2 genotype remains very interesting given the peculiar sweet taste and the pronounced red colour of the fruit and should have more attention in apple breeding programs and cultivar extension. Dessert quality is not the only consideration in advancing breeding selections. Desirable tree form, yield and fruit storability remain important. Actual context of climate change should be taken

into account in new apple breeding programs. With global warming, extending apple tree with low chilling needs in mild climate zones is the topical challenge for local strategies of apple production. Assessment of the 13 hybrids on the basis of quality traits of the fruit showed several promising cultivars for in-field extension. The present study will be continued by monitoring chilling needs of the tree in the orchard and storability of the fruit at the postharvest stage.

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