UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Institute for the Preservation and Improvement of Valencian Agro-diversity

Comparative study between advanced selections of raspberries (Rubus idaeus L.) under different crop management systems considering the most important phenotypic components for yield and fruit quality

Master's Thesis

European Master Degree in Plant Breeding

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ACADEMIC YEAR: 2021/2022

Universitat Politècnica de València

G-Berries S.r.l.

"Comparative study between advanced selections of raspberries (Rubus idaeus L.) under different crop management systems considering the most important phenotypic components for yield and fruit quality"

Erasmus Mundus Master's degree in Plant Breeding

2020-2022

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September 12th, 2022

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ACKNOWLEDGEMENTS

This thesis, as a closing academic work for the master's program, is manlily dedicated to my family that has been always present and supported me to grow as a professional and be the best that I can doing what I love.

I would like to extend my sincere thanks to European Master Program in Plant Breeding for the opportunity of getting into the plant breeding business. To all the consortium and professors from UniLaSalle Polytechnic Institute and Universitat Politècnica de València for their support in the academic and also personal and professional areas. Being part of this program has been completely a life-changing experience, as a professional and as a person.

I must also thank Molari Società Agricola S.S. and G-Berries, particularly Matteo Molari and Luca Molari for letting me learn about raspberries and being part of the team, giving me the chance of applying my skills, and also helping me to improve every day.

Special thanks to Professor Carmelo Lopez Del Rincon, my tutor from the UPV, for helping me during this thesis's administrative process and always being present with a good attitude to help.

I gratefully acknowledge my classmates, they became a family to me during these two years, supporting me every day, and they are special people from whom I learned so many valuable things.

ABSTRACT

Phenotyping is one of the most challenging steps when developing a new plant variety. It has been reported that there is an important interaction between genotypes and environment in the genus *Rubus* L. Considering the expression of important traits in different areas of production and under different crop management systems is crucial to understand the development and the potential of a new cultivar. New red raspberry (*Rubus idaeus* L.) cultivars are focused on the ability for double-cropping production, which means to have fruits on current-season canes and also produce additional fruit the following year as a second year. In addition to this production capacity, the main traits to achieve in raspberries are related to the production period, the architecture and growth habit of the plant, disease resistance, fruit quality, yield per plant, and ease of picking of the fruits. All these attributes are aimed to be improved to enhance crop management and increase the benefits for the growers. This work aims to define and validate a phenotyping protocol to describe the performance of two different advanced selections under three different growing fields in a potted farming system under high tunnels during the second-year production, considering a commercial variety as a benchmark. The major qualitative and quantitative traits influencing crop development, yield, and fruit quality were observed and evaluated during this assessment to understand which are the most suitable traits for cultivar description and which are the ones considerably influenced by agronomic management. The evaluation during this study was based on phenotypic data and temperature records, morphological characteristics of the plants, and sensory evaluation of the berries harvested during the summer season. The main differences considered between the three growing fields were the location, pruning system, and greenhouse structure. Phenotyping protocol was based on the UPOV (International Union for the Protection of New Varieties of Plants) protocol for the registration of new varieties and specific protocols developed for fruit evaluation based on cited bibliography. Collected data were analyzed and the conclusions allowed describing each genotype for its agronomic development and potential production based on yield components and fruit quality parameters. The weight of the fruit and the content of soluble solids turned out to be the main parameters influenced by the place of cultivation, while the sensory profile and the quality of the harvested berries allowed accurately characterize each genotype. From the results obtained, one of the selections presented optimal behavior for the production of marketable raspberries with a good competitive level, while the other selection did not reach the parameters to be considered as a potential cultivar for summer production from one-year-old plants.

KEY WORDS: breeding, raspberry, phenotyping, trials, environment, selections, yield, quality.

RESUMEN

El fenotipado es uno de los pasos más desafiantes al desarrollar una nueva variedad de plantas. Se ha informado que existe una interacción importante entre los genotipos y el medio ambiente en el género *Rubus* L. Considerar la expresión de las características más importantes en diferentes áreas de producción y bajo diferentes sistemas de manejo del cultivo es crucial para comprender el desarrollo y el potencial de un nuevo cultivar. Los nuevos cultivares de frambuesa roja (*Rubus idaeus* L.) se centran en la capacidad de producción como doble cultivo, lo que significa tener frutos en plantas de la temporada actual y también producir frutos adicionales el año siguiente como segundo año. Además de esta capacidad de producción, las principales características a lograr en frambuesa están relacionadas con el período de producción, la arquitectura y el hábito de crecimiento de la planta, la resistencia a enfermedades, la calidad de la fruta, el rendimiento por planta y la facilidad de recolección de los frutos. Todos estos atributos están destinados a ser mejorados para mejorar el manejo del cultivo y aumentar los beneficios para los productores. El objetivo de este trabajo es definir y validar un protocolo de fenotipado para describir y comparar el desempeño de dos selecciones avanzadas diferentes en tres campos de cultivo en un sistema de cultivo en maceta bajo túneles durante el segundo año de producción, considerando una variedad comercial como referencia. Durante este estudio se observaron y evaluaron las principales características cualitativas y cuantitativas influyentes en el desarrollo del cultivo, el rendimiento y la calidad de la fruta para comprender cuáles son los rasgos más adecuados para la descripción del cultivar y cuáles son las que tienen una influencia considerable en el manejo agronómico. La evaluación se basó en datos fenotípicos y registros de temperatura, características morfológicas de las plantas y evaluación sensorial de los frutos cosechados durante la temporada de verano. Las principales diferencias consideradas entre los tres puntos productivos fueron la ubicación, el sistema de poda y la estructura del invernadero. El protocolo de fenotipado se basó en el protocolo UPOV (Unión Internacional para la Protección de Nuevas Variedades de Plantas) para el registro de nuevas variedades y protocolos específicos desarrollados para la evaluación de frutos basados en la bibliografía citada. Los datos recolectados fueron analizados y las conclusiones permitieron describir cada genotipo por su desarrollo agronómico y producción potencial con base en componentes de rendimiento y parámetros de calidad de fruto. El peso del fruto y el contenido de sólidos solubles resultaron ser los principales parámetros influenciados por el lugar de cultivo, mientras que el perfil sensorial y la calidad de las bayas cosechadas permitieron caracterizar con precisión cada genotipo. De los resultados obtenidos una de las selecciones tuvo un comportamiento óptimo para la producción de frambuesas comercializables con un buen nivel competitivo, mientras que la otra selección no alcanzó los parámetros para ser considerada como potencial cultivar para la producción de verano a partir de plantas de un año.

PALABRAS CLAVE: mejora genética, frambuesas, fenotipado, ensayos, ambiente, selecciones, rendimiento, calidad.

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List of abbreviations

ANOVA	Analysis of Variance
CATA	Check all that applies
CIE	International Commission on Illumination
CSO	Centro Servizi Ortofrutticoli
GDH	Growing Degree Hours
GxE	Genotype per Environment
HSD	Honestly Significant Difference
N/A	Not Applicable
NS	Non-Significance
PCA	Principal components analysis
SS	Soluble Solids
TA	Titratable acidity
UPOV	International Union for the Protection of New Varieties of Plants

1. INTRODUCTION

1.1. Economic importance

The red raspberry (*Rubus idaeus* L.) is a high value fruiting crop worldwide that has recently increased its importance in terms of level of production and quality, with a production more than doubling in the last three decades (FAOSTAT, 2020). European production is dominated by Poland, a country that specializes mainly in exporting frozen fruit. With production that is aimed at export, Spain is the top supplier of fresh raspberries within the European Union for which there is a growing demand. Demand for this berry is taking higher importance in Europe and other continents, mainly due to the improvements released in the last years related to the shelf-life, fruit quality, and the progresses in refrigerated transport and storage, but also due to perceived health benefits (Graham & Brennan, 2018).

The growing area for fresh berries has a growing trend in Italy, although at a slower rate than the increase in consumption. The Italian supply is still limited in relation to the growth in consumer demand, so there is still scope for increasing the area in the short term. Growth is significant for blueberry, but other species such as raspberries, blackberries and currants are also tending to increase. According to the CSO (CSO ITALY - Centro Servizi Ortofrutticoli), the upward trend of recent years has continued in 2021, with an increase of 10% over 2020 with almost 2,200 hectares of berries in Italy as a whole. Raspberries have the second place in order to importance, having an increase to around 400 hectares, with an increase in production especially in the South, while in the North the figures were more or less stable, especially in the main production regions such as Trentino Alto Adige, Piedmont, Veneto, Lombardy, and Emilia-Romagna (Figure 1).

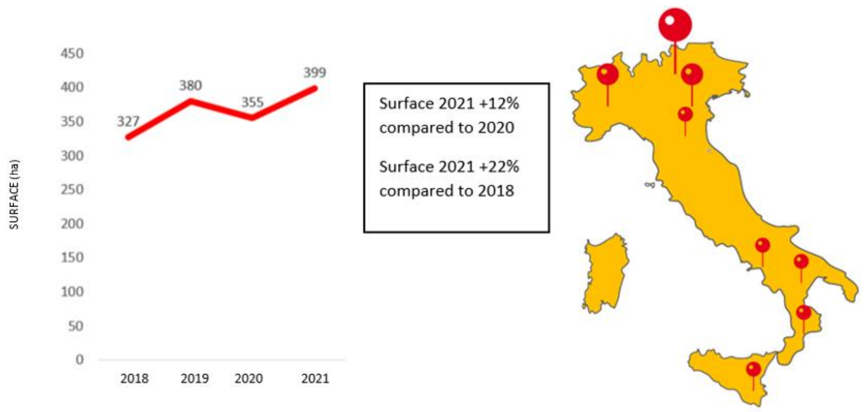

Figure 1 Raspberries' growing area in Italy. Left: Raspberry area trend 2018-2021. Right: Larger areas currently in Trentino, followed by Piedmont, Veneto, Lombardy, and Emilia-Romagna. Reproduced from CSO ITALY (Centro Servizi Ortofrutticoli

1.2. Botany and Morphology

The European red raspberry, *Rubus idaeus* L., is a temperate fruit crop belonging to the Rosaceae family (Table 1), included in the genus *Rubus* L., in the family Rosaceae (Funt & Hall, 2013). The Rosaceae family contains many other fruit crops of economic importance in the temperate regions of the world: apple, pear, sweet and sour cherry, peach, plum and strawberry. The genus comprises of a highly diverse series of over 700 species with a chromosome number of x = 7 and ploidy levels ranging from diploid to dodecaploid (Graham & Jennings, 2009). Members of the genus can be difficult to classify into distinct species for several reasons including hybridization between species and apomixes, but molecular studies are now assisting to understand and describe the phylogeny for Rubus (Graham & Brennan, 2018). Rubus includes five subgenera (I*daeobactus, Cylactus, Anoplobactus, Chamaemorus* and *Malachobatus*) that have the fruit character of a raspberry-type. The most important varieties are included in the subgenera *Idaeobatus* and it is where the main developments has been done until the last years. Cultivated cultivars contrast significantly from the original wild types by having larger and sweeter fruits, better adapted to travel to different markets and full fill consumer's preferences (Funt & Hall, 2013).

Table 1 Taxonomic classification. Data obtained from Integrated Taxonomic Information System (ITIS)

Family	Rosaceae	Roses
Genus	*Rubus* L.	Raspberries, blackberries
Species	*Rubus idaeus* L.	Common red raspberry, western red raspberry, American red raspberry
Variety	*Rubus idaeus var. idaeus* L.	European red raspberry

As described by K.Humer et. al. (2013), by morphological definition, raspberry fruits consist of clusters of small drupe fruits called drupelets. The aggregate of drupelets is held by interlocking hairs and the hole group of them separate as a unit fruit from a conical receptacle when the ripe fruit is picked.

Stephens, (2012), describes the morphology of the plants as deciduous perennial shrubs that need trailing to erect canes, where the canes are typically biennial, and the roots are perennial. Leaves are alternate pinnate with usually five leaflets on the primocanes and three leaflets on the laterals (second-year growth). Petioles and petiolules usually resemble the canes, and stipules are always present at the base of the petioles. The flowers are hermaphroditic; however, in some cases, they are unisexual, especially in the wild (Badenes & Byrne, 2012). Canes can be glabrous, hairy, or thorny. Cultivars can be divided into "floricane-fruiting" and "primocane-fruiting" types. In floricane-fruiting cultivars the canes are kept for two years. During the first year the canes that are called primocanes grow vegetatively until floral induction in the fall. In the second year, lateral branches grow from the nodes of canes, and these are the responsible of producing flowers and the fruits (Palonen et al., 2021). After fruiting, the canes die to be replaced by the new canes to continue the plant cycle. Some genotypes are able to fruit only on the first-year canes and other produce on both type of growing seasons having the name of remontant or everbearing cultivars (Graham & Brennan, 2018). Morphology of a raspberry plant is illustrated on figure 2.

Flower induction is the main physiology to understand to describe raspberry production. Palonen et al. (2021), explains that primocane-fruiting cultivars initiate flowers regardless of day length and temperature, while in floricane-fruiting types, flowers are initiated under short days and low temperatures. In effect, the fundamental difference between these two types of cultivars is that, in floricane-fruiting cultivars, floral initiation is followed by the onset of bud dormancy, whereas in primocane-fruiting cultivars flower development directly follows floral. However, the separation between these two different life cycles is not distinct; and each cultivar can behave in a different way, some genotypes allow double cropping of the primocane varieties, since the upper part of the cane produces crop on a primocane, while the lower part behaves like a floricane variety, other did produce only on the one-year-old cane and others have the main production on the primocanes (Hanson et al., 2019).

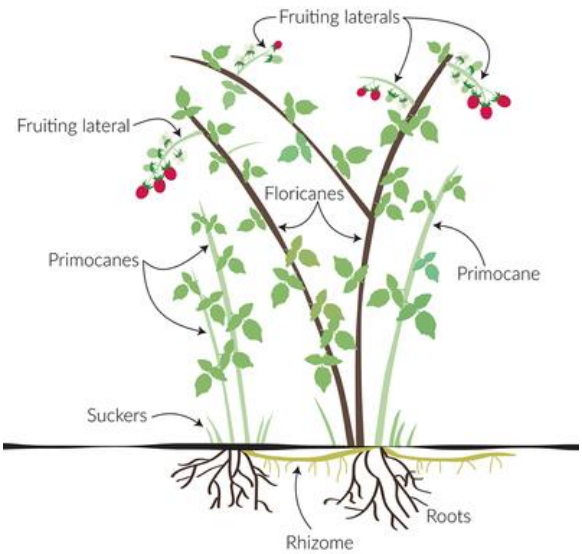

Figure 2 Morphology of a raspberry plant. Vegetative and fruiting organs of the plant. Reproduced from University of Minnesota resources (https://extension.umn.edu).

1.3. Cultivation and fruit production

During the past ten years, fresh red raspberry production has increased dramatically as a result of improvements in production methods and year-round consumer demand for fresh, high-quality fruit (Badenes & Byrne, 2012). To meet consumer demand, various systems for improving yield and expanding the season of berry production have been developed. Substrate pot culture of raspberries grown in high tunnels has the potential for greater yields than traditional in-ground soil production (Hanson et al., 2019).

Protected cropping in high tunnels and out-of-season production for fresh market across the European countries are increasingly expanding. The production is conducted under polytunnels of varying design (Figure 4), to give optimal growing conditions and the ability to extend the cropping season beyond the traditional short summer period. Under tunnel production, raspberries are protected from rain and wind damage, reducing the prevalence of abiotic stresses (Hanson et al., 2019). Nevertheless, tunnels have the potential to reduce light intensity and increase air and soil temperatures which could negatively influence quality raspberry production in warm summer months (Nestby et al., 2012).

In addition to growing under covered structures, pot culture (Figure 3) provides advantages for manipulating the timing of fruit production by keeping plants in cold storage until the desired production time (Nestby et al., 2012). Furthermore, growing plants in pots in soilless media avoids soil limitations such as pathogens, which may be present in any given field location (Hanson et al., 2019).

Figure 3 Potted long cane plants with irrigation system and guides to support the fruiting laterals. Picture taken at the experimental field.

Potential benefits of potted plants are mitigated by the costs associated with pots, substrate, complex irrigation requirements, and the labor needed. The key elements of these intensive systems, in addition to the protected growing facilities, include the use of high-quality plant material. The new cultivation challenges require to move forward for development of cultivars with new and stable adaptations. Fruit size is considered especially important in relation to production costs. As raspberry harvesters are paid an hourly income, making larger fruit more economically viable, as a higher weight can be harvested in the same time frame (Pinczinger, 2022).

Figure 4 Growing tunnel structures with potted raspberry plants. Picture taken at the experimental field.

The fruit production and quality level in raspberry are the main challenging traits. The objective is to harvest a large firm, conical-shaped berry with high sugars and contrasting acidity, which makes the fruit agreeable for the consumer (Figure 5). The expected color for the fresh market is a bright, shiny red that does not darken in storage, while for processing, only uniform dark red is required (Funt & Hall, 2013).

Figure 5 Raspberry fruits with fresh market qualities. Big size conical fruits ready for the picking. Picture taken at the experimental field.

Different production calendars can be adopted by growers, depending on the area of production and the market. One harvest per year from the one-year-old canes, during early summer, another option is to stablish the plants and have the production on the same yar from the primocanes in autumn, changing the plant material every year. Another option is to have to harvest per year, starting with the first primocane and maintaining the cane for the next summer production, picking fruits from the primocanes and then from the floricane (Graham & Brennan, 2018). Cultivars, depending on their characteristics, can be more or less suitable for each management. In floricane-fruiting cultivars the canes are kept for two years. During the first year, primocanes grow vegetatively until floral induction and they fruit usually late in the fall. The over winter canes (floricanes) produce fruit in the summer of the second year and high yields with excellent fruit quality can be obtained from both harvests (Palonen et al., 2021). The growing system adapted by growers defines the plant material needed from nurseries. Long cane plants (one year old canes) that are used in planting especially in high tunnel and substrate culture, are plants of floricane cultivars with initiated flower buds, lifted and placed under cold storage at the end of their first year. On the other hand, for a primocane growing design, small fresh plants are the starting plant material (Carew et al., 2000).

Double cropping increases total yields of several primocane cultivars in a short growing season area, many of the newly released raspberry cultivars are primocane-fruiting and have high yield potential as well as excellent berry quality. One option to overcome the challenges of a short growing season is to grow the promising primocane varieties as floricane varieties in a biennial system for summer cropping (Hanson et al., 2019).

1.4. Breeding in raspberries

Breeding in raspberry is carried out by hybridizations between cultivars or species with desirable characteristics for multiple generations. After germination of the obtained seeds, plants are grown in tunnels and evaluation of vegetative and productive traits are evaluated, following a scheme of clonal selection (Graham & Brennan, 2018). As a vegetative propagated crop, spontaneous mutations or soma clonal variation through generations has been reported as a source of genetic variation and also some cultivars are a result of these phenomena (Graham & Jennings, 2009).

Although most of the produced raspberries worldwide are processed, for the frozen, jams and sauces industry, there has been an increasing demand for fresh raspberries and growers are demanding varieties able to produce in larger periods of the year and cover periods considered “out-of-season”. Producers are also interested in growing high quality fruiting raspberry cultivars for the local market as well as for long shipping. The expectations from the final consumers are focused on finding good quality berries on retailers' shelves, is challenging the fruit quality and shelf life of the raspberry varietal development (Badenes & Byrne, 2012).

An important trait that should be further explored in relation to fresh fruits is the flavor. Flavor of raspberries can be a divisive topic as described by different specialists of the industry. Traditionalists often prefer a bit of bite and acidity in their raspberries, but younger palates may prefer sweeter berries, as with other crops and food. Actual breeding programs are in the search of the perfect balance in flavor. Consumer liking has given high importance to include panel test protocols during varietal development, to allow breeders understand what consumers' response would be to different raspberry varieties (Graham & Brennan, 2018).

Another challenge, of current breeding programs is to transfer traits of interest, especially, good plant structure, environmental adaptation, disease, and pest resistance, from the available material in order to improve old cultivars, leaving deleterious traits behind (Funt & Hall, 2013) while improving fruit quality and production.

1.4.1. Fruit quality

Fruit quality is defined as the level of excellence and the perception of conditions acceptable to consumers. It is an important parameter to most buyers, yet it is one of the most complex and uncertain factors that producers, breeders, and other fruit researchers must consider (Morel et al., 1999). These perceptions are the attributes which give value to the fruit as evaluated by the perception of a final customer. Attributes include edible quality, nutritional quality and also export quality. Therefore, quality always depends on the intended use (e.g., fresh or processed) and covers a range of traits. Berry size, freshness, color, firmness, and shelf-life are considered as physical properties while sweetness, sourness, flavor, and nutritional composition are chemical properties (Brennan and Graham, 2009).

High yield, good appearance, easy to harvest and long shelf-life for distance shipping are the quality traits perceived by the growers while freshness, firmness, size, color, and flavor are quality characteristics considered by most consumers. Vitamins, minerals, dietary fibers, and many bioactive compounds are considered to be nutritional qualities (Alibabic et al., 2011).

Raspberry fruit quality depends mainly in three factors: cultivar, environment, and agronomic management as in many other fruit species (Badenes & Byrne, 2012). Light and temperature are the main environmental factors affecting berry quality before and post-harvest. For example, the effect of high temperatures during the post-flowering period has been reported by Nestby et al. (2012) as a crucial factor influencing the development of the berry, reducing the final berry weight. According to bibliography, fruits grown in warm and dry summers are sweeter, less acidic, more aromatic and have darker color (Prits, 2013). Furthermore, it has been described that temperatures greater than 30 °C reduces the aroma of the fruit and wet conditions reduce the sugar content. Jennings and Carmichael,

(1980) reported that berries were more aromatic in warm and dry conditions than in mild and humid conditions.
The temperature under high tunnels is warmer than in open field conditions but light exposure is reduced which decreases total anthocyanin in raspberries. The reduced light under the high tunnels affects the different components responsible for the color and lighter fruits can be harvested (Kassim et al., 2009).

Information about the physicochemical properties and morphology characteristics of raspberry fruit is essential for understanding the potential growing system of each variety. Fresh raspberries have a very short shelf life and are generally only readily available around summer, which means that postharvest value is one of the most important traits to consider when it comes to develop a new cultivar (Nestby et al., 2012). For physical quality, the size of berry is a key objective in many breeding programs, as this attribute can have a significant impact on the cost of harvest. Shelf-life and fruit softening also significantly impact costs in production due to losses on farm and rejects from retailers. Fruit softening is an important agronomical trait that involves a complex interaction of plant cell processes during maturation. The main aspects to be studied and that make varieties be differentiated are the color, fruit weight, caliber and shape, content of sugars, acidity, and the balance between these two lasts. Also, the general aspect of the berry should be considered as fruit quality, including absence of broken drupelets and absence of any disease (Graham & Brennan, 2018).

1.4.2. Sensory evaluation

Sensory methodologies are used to define the commercial potential of pre-breeding material, new cultivars, and innovative production techniques by assessing sensory attributes and consumer appreciation (Aaby et al., 2019). Sensory profiles produced by trained panelists can identify strengths and weaknesses of the quality of a genotype, improving the efficiency of selection steps during a breeding program. Advanced methods applied in consumer tests are useful for identifying preference drivers to promote the selection of pre-commercial material and provide tools for decision-making about cultivar release (Lado J et al., 2019).

Fruit quality is the main aspect for consumers of fresh fruits. Sensory properties of raspberries comprise appearance, odor, flavor, and texture, which together determine the attractiveness of the berries (Lado J et al., 2019). These characteristics are determined by the chemical composition of the fruits. Anthocyanins are responsible for the red-purple color of raspberries. Flavor is defined by taste and odor-active compounds, are detected by the olfactory system. Sugars and acids are the main taste compounds in raspberries, but also phenolic compounds, for example, may contribute to bitter taste and astringency influencing the perception in mouth. Fructose, glucose, and sucrose give raspberries their sweet taste. The raspberry aroma is due to a mixture of odor-active volatile compounds, which can vary in proportion and concentration giving different aromatic profiles to the different cultivars (Alibabic et al., 2011).

A complete and objective way to determine sensory quality is a descriptive analysis conducted by a sensory trained panel combined with physicochemical measurements of the main components. Therefore, there is an aim to identify chemical compounds and instrumental measurements that correlate with sensory attributes and thereby can be used to predict sensory quality. Aaby et al. (2019) have published a protocol for sensory and instrumental protocol for raspberries selections. Color can be determined by the CIE (International Commission on Illumination) color system by instrumental analysis, or by using standardized color charts. Sweet taste is assumed to correlate with sugar content, which easily can be determined as soluble solids (SS) and acidity is influenced by contents of organic acids and can be determined as titratable acidity (TA). Contents of sugars and acids, determined by simple measurements of TA and SS, and especially the SS/TA ratio, correlated well with important sensory attributes such as sweet taste, acidic taste, and astringency.

In sensory 'trained panel' studies using descriptive analysis, panelists often use a scoresheet, with each variable's assessment measured and recorded on a continuous line scale, by drawing a mark on the scale at the appropriate point (Lado, 2019).

To describe consumers preferences, the application of CATA "check-all-that-apply" questions can be a useful tool which involves assessors selecting all terms that apply to a product from a list of descriptors or associations (Lado et al., 2010). CATA has been shown to be reliable and stable for sensory product characterization by consumers and to produce results that are similar to traditional descriptive analysis with a trained panel. CATA has been used in studies to identify ideal products, in order to list the characteristics of assessors' ideal product, which is a simpler and more intuitive task than rating ideal intensity in classic ideal product profiling (Ares et al. 2011). Again, it has the advantage that consumers can be used to identify ideal product profiles without the need for trained panel descriptive analysis. Several studies show results in which they were able to identify positive and negative hedonic drivers, which can be used for optimization.

1.4.3. Yield components

Yield is considered as important as fruit quality when it comes to list the main objectives of raspberries' breeding (Stephens, 2012.). Yield is linked with the improve of management, as a prerequisite of high yields is excellent adaptation to the environment. This includes the ability to grow and yield under the abiotic conditions of substrate, temperature, and humidity and the biotic stresses in the production zone (Badenes & Byrne, 2012).

Main yield components, shown in Figure 6, are cane number, cane length, cane diameter, lateral number, lateral length, fruit numbers per lateral and fruit size (Stephens, 2012). Depending on the fruiting cycle of the cultivar yield in defined by different traits. In summer-fruiting types, the most important plant characteristics include number and height of young cane, consistency of bud break, internode length and lateral length and position. In primocane-fruiting types, the amount of branching and extent of lateral development on the primocanes are major yield components. In both types erect, thornless canes are desirable (Graham & Jennings, 2009).

Final berry value, considering quantity and quality in fresh raspberries is highly affected by the preharvest hanging ability of the laterals, the post-harvest shelf life, and the resilience under diverse environmental stresses such as temperature, pest, and diseases (Nestby et al., 2012).

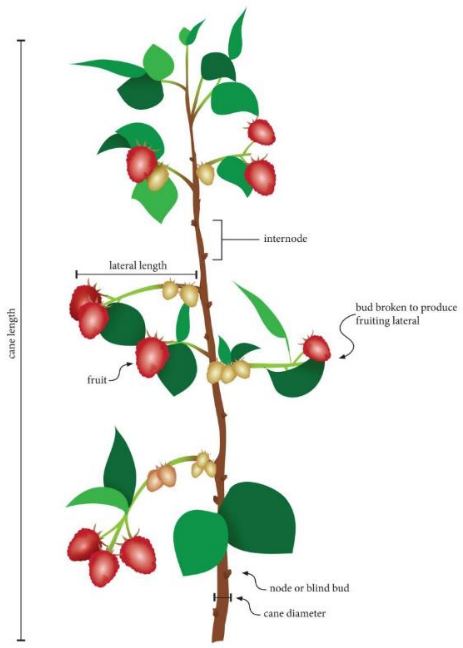

Figure 6 Single raspberry fruiting floricane showing yield components. Reproduced from bibliography (Stephens, 2012).

1.4.4. Phenotyping

Considering phenotypes are a result of the interaction between the genotype and environmental factors during all the plant life cycle. As a role of a plant breeder, it is to evaluate such crop phenotypic traits to obtain data and characterize different phenotypes and be able to estimate key plant development stages useful for selection and management decisions. Thus, quantifying plant phenotypes associated with different plant genetics under diverse environmental circumstances can improve the understanding of genotype-environment interactions. This concept is known as applied for plant variety selection in standard crop breeding schemes.

The rise in phenotyping technology is providing breeders and agronomists with the ability to assess exactly what is happening at the whole plant and subsoil levels, so that cultivars can be oriented to specific production sites and managed more efficiently for maximum crop yields and enhanced quality (Graham & Brennan, 2018). Field observation and evaluation is a common approach during the description of new genotypes, which allows identification of agronomically important traits and also the development of selection criteria for breeding. The high impact of environmental conditions and plant development stage make a high impact on the expression of the genotype of each individual (Lācis et al., 2017). The key factor in understanding traits at a genetic level is the capability to correctly phenotype. The main limitation usually is the significant effort and complexity of measurements, depending on the trait, required to obtain phenotyping data from plants grown in appropriate environments (Graham & Brennan, 2018).

In a breeding program, the evaluation and selection of interesting material includes data of the plant phenology and morphology attributes. Phenology statements in raspberries are essential to realize the most suitable production cycle for the plant and also this knowledge allows to understand the most suitable environment to grow it matching the expectations of the grower, the market, and the requirements of the cultivar. On the subject of morphology there are two areas of description, the physical characteristics to describe a plant that allows to make it differentiable from others of the same genus and the attributes that are related to production in terms of quantity and quality (Alibabic et al., 2011).

The attributes of the fruit, including chemical and sensory characteristics, are the final expression of the interaction between genotype and environment (Malosetti et al., 2013). This phenotypic expression highly varies among cultivars and in between the same genotype the final quality of the berry depends on many factors, such as environmental factors (temperature and humidity), substratum, irrigation, yield efficiency, ripeness of harvested fruits, the general agronomic management (Hudson, 1959).

Since the main objective to breed raspberries is the increasing of good quality production and making the production period longer (Badenes & Byrne, 2012), the focus is onto the evaluation of double cropping genotypes. Most of the last raspberry cultivars are primocane-fruiting and have high yield potential as well as excellent berry quality. But the global production of this fruit includes also short growing season, which means only one harvest period in one year. This production system also aims to have high quality and high yield cultivars. One option to overcome the challenges of a short growing season is to grow the promising primocane varieties as floricane varieties in a biennial system for summer cropping. Considering this challenge, it is worth to evaluate promising primocane varieties as floricane ones and its potential for summer production (Palonen et al., 2021).

Raspberry quality shows a significant inter and intracultivar variation, revealing the great impact of the genotype, environmental and cultivation practices on quality and sensory characteristics (Hudson, 1959). Moreover, fruit to fruit variation has also been reported, which impacts physicochemical quality and fruit flavor. Therefore, the development of new appealing cultivars capable of reducing quality fluctuations associated with environmental factors or management strategies is of high interest (Birgi et al., 2019).

1.5. The region

In Italy there are various areas where soft fruit is cultivated successfully. According to the information from the site of "Centro Servizi Ortofrutticoli", Trentino is the main production area, followed by Verona and Piedmont. Also, the cultivation in the south is growing and the main areas are Calabria and Sicily. Romagna is not the main region, but there are also growers investing in berries, mainly focused on local fresh market (Figure 1).

Emilia-Romagna Region is one of the leading agricultural regions in Italy with more than one million hectares of farmland, of which 79% is used for arable cropping, 10% for pastures and permanent grassland and 11% for permanent crops. Cereals, fruit, vegetables, and grapes (for winemaking) are the main crops and about one third of the region's farms have some irrigated land. About 75% of all farming in this region is of high and medium intensity. The region is well known for producing fruits for the fresh market such as peaches and pears.

The province of Forlì-Cesena (Lat: 44° 7' 59.9916''N - Long: 12° 13' 59.9988''E) presents warm, humid, and mostly clear summers, and cold and partly cloudy winters. Over the course of the year, the temperature typically varies from 32°F (0°C) to 85°F (30°C) and is rarely below 24°F (-4°C) or above 92°F (33°C). The hot season lasts for 3.2 months, from June 6 to September 13, with an average daily high temperature above 77°F (25°C). The hottest month of the year in Cesena is July, with an average high of 84°F (29°C) and low of 64°F (18°C). The cold season lasts for 3.4 months, from November 21 to March 3, with an average daily high temperature below 52°F (11°C). The coldest month of the year in Cesena is January, with an average low of 32°F (0°C) and high of 45°F (7°C) (Figure 7 and 8).

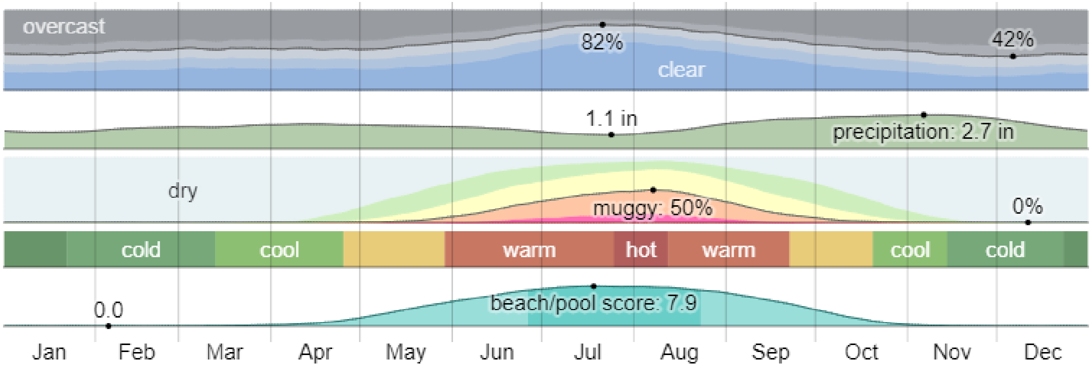

Figure 7 Forlì-Cesena weather by month. Reproduced from © WeatherSpark.com

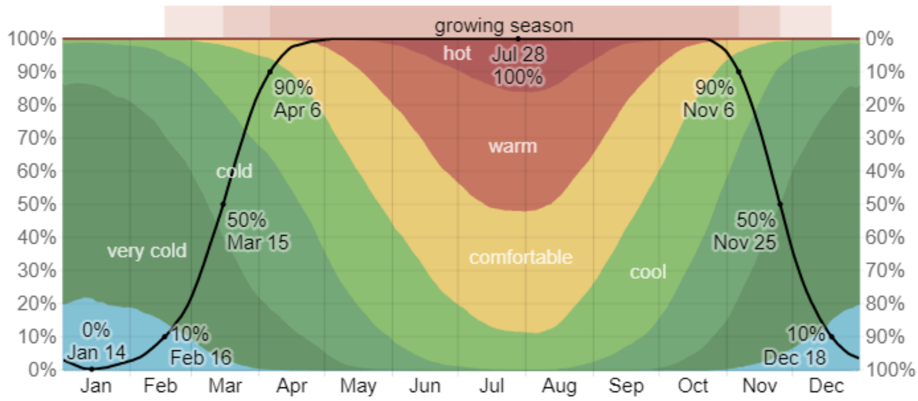

Figure 8 Forlì-Cesena growing season. The percentage of time spent in various temperature bands. The black line is the percentage chance that a given day is within the growing season. Reproduced from © WeatherSpark.com

1.6. This study

The purpose of this study is to evaluate the production and fruit quality of two advanced raspberry primocane fruiting selections during summer production. The evaluation will be carried out by doing a comparison of the different phenology periods, yields, and fruit quality of the selections under different agronomic managements, including different pruning system, Fert irrigation, and tunnel structure, with the objective of understanding better the development of the plants and their behavior in comparison to a benchmark variety "Enrosadira" (EU PVR 48949, Plant Patent USA 14120026, PBR Australia 2017050). Enrosadira™ has an early season and it is highly productive both in primocane and floricane cropping variety. In Northern Italy, the cultivar starts cropping in late May for the floricane crop and in late July for the primocane the berries have a bright light red color, they have a good aromatic flavor, and they present good firmness and shelf life. The cane has very vigorous growth with small lateral branches and small spines. Lateral branches increase after the plants have been cut. Enrosadira™ is tolerant to the main raspberry pests & diseases.

Influence of the genotype, the environment and interactions between genotype and environment on different traits of the plants in the main objective to observe during the production season. It is expected to observe how the different reactions of genotypes varies in dependence on the environment (Malosetti et al., 2013). Based on the statistical package reported by (Snyder & Melo-Abreu, 2005), the evaluation of climate influence on fruit production is critical for decision-making in the design stage of agronomic production systems, and in the evaluation of the potential consequences of future climate. Concerning environment of growing fields, climatic indices and plant phenology are commonly used to describe the suitability of climate for growing quality fruit and to provide temporal and spatial information about regarding ongoing and future changes. The statistical package, 'fruclimadapt' updates the assessment of climate adaptation and the identification of potential risks mainly for fruit trees. This allows to calculate growing degree hours (GDH), a measure of heat unit accumulation that accounts for seasonal differences in development time. The basic concept in heat unit accumulation is that growth occurs when temperatures are above some base temperature and that growth rate increases with temperature, until it reaches an optimum temperature, at which there is no longer a continued increase in growth rate, whereas the critical temperature is the maximum temperature at which growth will continue. The heat units required to promote flowering have been reported to vary dramatically by genotype indicating significant genetic variation (Black et al., 2008).

This analysis should give tools to carry out a phenotypic description and understand which are the traits that allows to differentiate the varieties, and which are the ones that are more affected by the environment. As a consequence, also, the identification of the adaptability of the future varieties to the management of different growers will be evaluated. The selections have been already selected because of their primocane potential, but with this evaluation the objective is to define their capability to double cropping, observing, and analyzing their floricane production.

2. OBJECTIVES

1. Define a phenotyping protocol suitable for raspberries.
2. Describe the phenology and main morphological characteristics of each selection.
3. Define the most important qualitative and quantitative traits related to yield and fruit quality.
4. Define the traits that allow to differentiate between the cultivars due to the genetics and the ones that are influenced by the agronomic management.
5. Compare the performance of the selections and find the most suitable one for each management during the floricane production.

3. MATERIALS AND METHODS

3.1. Plant material and field trial design

The assessment followed a multi-environment trials experiment. An amount of ten randomized per genotype were stablished at the different locations for the present study (Table 2). Plants were stablished at each location during the year 2021, produced during the primocane season and were maintained over the winter for the floricane production.

Each site represents a commercial field for production of fresh raspberries. The three locations considered are three different tunnel structures in the Emilia Romagna region in Italy.

Table 2 Two-way table of genotype by environment

LOCATION / GENOTYPE	A	B	C
AMO-008	AMO-008-A	AMO-008-B	AMO-008-C
AMO-013	AMO-013-A	AMO-013-B	AMO-013-C
AMO-022	AMO-022-A	AMO-022-B	AMO-022-C

The differences between each location will be the tunnel structure and management, which generates different environmental conditions inside. Location A is considered as the control tunnel with a standard management, location B has a management focused on late production, while location C, promotes higher temperatures early looking for an early production. To have a later production, at location B the tunnel is uncovered during winter until the beginning of spring, and at the other two locations tunnels are covered the hole year. The structure C, is completely covered, generating higher temperatures at the beginning of the vegetative cycle.

The three cultivars under analysis are two advanced selections of a breeding program and one benchmark commercial variety. The materials were labeled as AMO_008 (benchmark), AMO_013 and AMO_022.

3.2. Data Collection

3.2.1. Morphological description

For the description of each genotype, characters regarding the structure of the plant from UPOV guidelines for the registration of new varieties were selected to describe and differentiate each raspberry selection and assure homogeny in between plants used for the production and quality evaluation.

3.2.2. Environmental conditions

To evaluate the correlation between the three different locations and the production and quality traits evaluated, temperature and humidity at each growing tunnel were quantified. During the hole season temperature and humidity were recorded at each location. Inside each tunnel one Elitech data loggers (ElitechLog V6.1.0) was installed, and it was settled for recording hourly lectures of both parameters. The data was downloaded by using the specific data management desktop software designed for analyzing and managing the temperature and humidity data recorded by Elitech data loggers (Figure 9).

From the date of budburst, hourly temperature records were used to calculate growing degree hours (GDH) at each location, to quantify the requirement for flowering and to fruit harvest for each selection at each location.

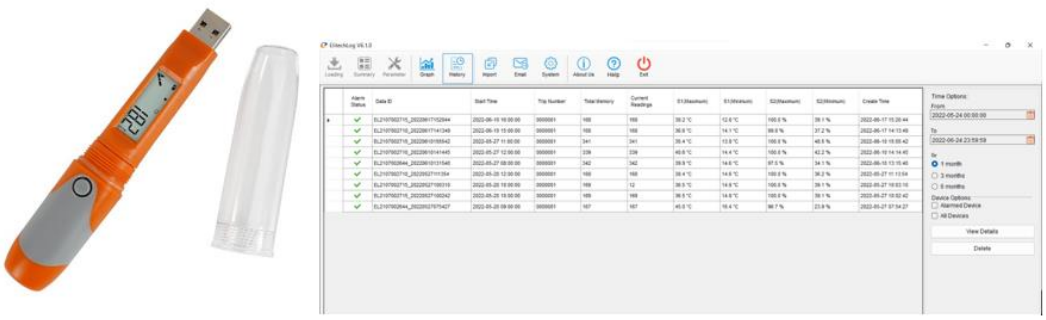

Figure 9 Elicitech datalogger and desktop software ElitechLog V6.1.0.

3.2.3. Phenology assessment

To evaluate bud burst, cumulative data of open buds was noted every week. As an open bud, it was considered the statement when green leaves can be distinguished.

Blooming was evaluated by the number of open flowers. Weekly registration was performed in order to build a flowering curve across the season for each selection at each location under study. Data for fruit maturity was registered as the harvest time started. During the harvest the beginning, the peak and the end of the period was used to describe the production of each selection.

3.2.4. Yield components

Data was measured on the one-year-old cane of each plant. Measurements of the number of buds per pot, length of the internodes, diameter of the cane un the upper, central, and lower part of the cane. Number of fruiting laterals, length of fruiting laterals and number of fruits per lateral. During harvest total weight of berries per day was registered and at the beginning, peak and end of the harvest individual fruit weigh was measured.

Fruits were picked three times per week. Harvest was done early in the morning, to avoid the high temperature of midday in 125gr boxes (Figure 10). The harvested fruits are collected separately in two groups: 1st category, and 2nd category. The second category fruits were injured fruits, such as deformed, crumbly fruits, with white drupelets, green tips, double fruits, and very small berries. The harvested fruits were then weighted in the laboratory and data was registered for each category group, to calculate the percentage of each category in the total production. After the registration of the weight, fruits were used for different evaluations. To complete the data collection regarding to yield, individual fruit weigh data was recorded.

3.2.5. Fruit quality

Soluble solids and titratable acidity were measured from the harvest fruits from the three locations and for each genotype. The analysis was done by three repetition and during three moments of the harvest period. Samples were taken at the beginning of the harvest, during the peak and at the end of the harvest period, in order to have representative results of the hole picking season. In order to determinate and value the shape and of the fruit, ten fruits were randomly selected by genotype from the first quality berries, at the beginning, pick and end of the season, to measure length and width, having in total the measurements of thirty fruits per genotype at each harvested field.

Fruits were measured using an electronic digital caliper (0-150mm) and a digital scale to weight the berries (Figure 10).

Figure 10 Materials used for harvest and fruit evaluation. Left: electronic digital caliper used for obtaining the measure of length and diameter of the berries; fruit from AMO-013 is shown on the picture. Right: Clamshells used for harvest. Picture shows first quality raspberries harvested from the selection AMO-022.

For each genotype three clamshells with first quality berries were destinated to the evaluation of the shelf life. Fruits for this evaluation were part of the harvest during the peak of the season. The parameters defined to evaluate this aptitude, were weight loss, SS, titratable acidity, color and the tendence to leakage. The determinations were done at day 0 (picking day), and after 4, 7 and 10 days of cold storage at 4°C. Berry color was evaluated by using The Royal Horticultural Society Color Chart (Figure 12). Leakage was determined counting the percentage of fruits presenting this defect. Each fruit was passed by a white paper and visual valuation was performed. General aspect of the fruits during cold storage was vaulted using a scale from 1 to 5, from bad to excellent conditions based on visual perception.

For the analytic measurements berries were frozen in order to quantify solid soluble (SS) and titratable acidity (TA). After defrosting, berries are placed in cheesecloth to extract the juice. With a pipette a sample of juice is placed on the automated refractometer Atago Refractometer PAL-1 (Figure 11), for SS measurement. For the acidity determination 1g of juice is diluted with 50 mL of deionized water. After the agitation a sample of juice and deionized water is placed on the automated refractometer Atago PAL-Easy ACID7 (Figure 11).

Figure 11 Instruments for solid soluble (on the left) and titratable acidity (on the right) determination.

Figure 12 Royal Horticultural Society Color Chart.

3.2.6. Sensory evaluation

To complete fruit evaluation different panel test were carried out to obtain correlations between sensory attributes and the chemical measurements. Each panel test was defined by the availability of high fruit quality and harvest period. Each sample was evaluated by ten panelists and the assessment included visual and sensory evaluation. With the objective of observing the response of the fresh fruit industry to a potential new variety, the benchmark cultivar, and the selections were tested under an organized panel test with professionals from the industry of berries, that have experience in doing sensory evaluation with raspberries.

The terms evaluated were color, uniformity, shape, glossiness as visual aspects, and taste, firmness, juiciness, sweetness, and acidity as palate aspects. Correspondingly, a scale of visual and taste preference was evaluated, under the term “liking”. The factors considered included the genetics, the place of cultivation and the post-harvest quality of the fruits, comparing the quality of the sensory attributes at the harvest day with five days after harvest, considering the consumers usually receive the product at this stage.

The sensory evaluation of the berries was completed by doing a panel test with consumer point of view. The survey included visual and taste evaluation and completed with a general perception of the fruit. Panelists had to answer a CATA (“Check all that applies”) question with 20 terms (Tale 3) related to sensory characteristics, based on the main quality attributes for fresh raspberries. They were asked to check all the terms that they considered appropriate to describe each raspberry.

For all the performed panel tests, raspberries were removed from the cold storage one hour before serving and were room-tempered $(20 \pm 2°C)$ at serving. The samples were prepared into small plastic cups labelled with random codes of letters to avoid the influence of the coding on the responses (Figure 13). The panelist received three berries per sample of first-class category, uniform size and without defects. Responses for the visual and taste evaluation were scored from 1 to 5 considering 1 as the lowest mark and 5 the maximum.

Figure 13 Samples for sensory evaluation. Three berries per sample presented in white glasses labeled with a randomized letter.

Table 3 Twenty terms proposed for the CATA analysis of the main raspberry's attributes for fresh fruit quality.

NOT VERY SOUR	NOT MUCH RASPBERRY ODOR	NOT MUCH RED COLOR	SMALL	NOT VERY JUICY
VERY SOUR	INTENSE RASPBERRY ODOR	INTENSE RED COLOR	BIG	VERY JUICY
NOT VERY SWEET	NOT MUCH RASPBERRY FLAVOR	REGULAR SHAPE	SOFT	NOT VERY BRIGHT
VERY SWEET	INTENSE RASPBERRY FLAVOR	IRREGULAR SHAPE	VERY FIRM	VERY BRIGHT

3.3. Statistical data evaluation

R software (R Core Team, 2020) was used for all the statistical assessments. Package "fruclimadapt" was used to apply the function "GDHasymcur" is used to calculate growing degree hours for each phenological stage for the three genotypes at each field and characterize each selection's requirements of temperature. Analysis of Variance (ANOVA) was carried out to detect significant differences in temperature records between the three growing fields. Significant differences were analyzed using Tukey tests ($p < 0.05$).

Data across the three environments were analyzed by Two-Way ANOVA ($p < 0.05$) to assess significance of Genotype, Environment, and Genotype × Environment Interaction. The variables evaluated were significant differences in production, morphology of the fruit (fruit length, fruit width, fruit weight), fruit quality variables (soluble solids and titratable acidity), shelf-life parameters and 11 sensory variables from the panel tests. Mean ratings were calculated, and honestly significant differences were checked using Tukey's test (P ≤ 0.05).

For the statistical evaluation of the data from the CATA test, for each checked attribute a "1" is assigned and a '0' if the subject did not check the attribute for the sample, the frequency of each term was used to do the statistical comparison. If an overall significant difference is found, an ANOVA is used a comparison technique to identify what product differences are responsible.

4. RESULTS

4.1. Morphological description

After a visual description of the three selections, description was made using UPOV characteristics and scales (View the characterization on supplement 1). The main differences that made the genotypes distinguishable are the presence and absence of thorns, the characteristics of the berries in terms of size and shape.

AMO-008 considered the benchmark for this study, has an early time of beginning of budding, beginning of flowering and fruit ripening. Erect growing habitus. Variety with small thorns, medium density. Greyish-brow winter cane presenting medium diameter, and medium internodes, overall medium-high vigor. Equal three or five leaflets along the plant, medium level of rugosity and dark-green color of the adaxial part of the leaf. Produces light red conical berries. Fruits are uniform, medium sized and weighted. The results correspond to the already existing varietal profile of Enrosadira™.

AMO-013 early variety, considering bud burst, flowering, and fruit ripening. Upright plant habit with thornless and medium vigor cane. Brownish-grey Winter cane color. Absent anthocyanin coloration on the apex, boom or hairiness on the cane or laterals. Medium-short length of internodes and variable length of laterals. Mainly three leaflets per leaf, dark green color of the upper side, absence of thorns on the pedicels, and weak rugosity of the leaves. Roundish small fruits, with dark red color, strong glossiness, medium firmness. Low integrity of the drupelets, medium adherence to plug, presence of white drupelets and no uniform shape.

AMO-022 late selection, starting each phenology statement ten days after the benchmark. Semi-upright habitus. Cane with thorns, low density. Small and purple spines present on current year's cane, winter cane and peduncles. Greyish-brow winter cane presenting thin diameter, and long internodes, overall low-medium vigor. Equal three or five leaflets along the plant, high level of rugosity and dark-green color of the adaxial part of the leaf. Produces orange to red, light colored conical berries. Fruits are uniform, big sized and high weighted.

No symptoms of diseases were observed, so the level of susceptibility is considered low, but no inoculation tests were done to be able to guarantee resistance.

4.2. Environmental conditions

The three growing field locations presented significant differences (alpha=0.05), for temperature, considering maximum values, minimum and average. The significant differences were between B and A, C and B, considering A and C not statistically different. On figure 14 two groups can be distinguished based on range of maximum temperatures. Location A had a mean maximum value of 29.5°C, location C 30.7°C and location B 26.11°C. Location B presented the lowest values, but also a smaller range of temperature during the day. Locations A and C presented larger range of values in between minimum and maximum temperature.

Location B presented the lowest value for minimum temperature of -5.9°C during the month of march, and maximum values of temperature did not reach more than 45°C, the average temperature during the production period was 40°C. Location C presented the higher temperatures, since the month of march it registered maximum values higher than 40°C. Minimum temperatures at location C only went under 0°C on March (Figure 15).

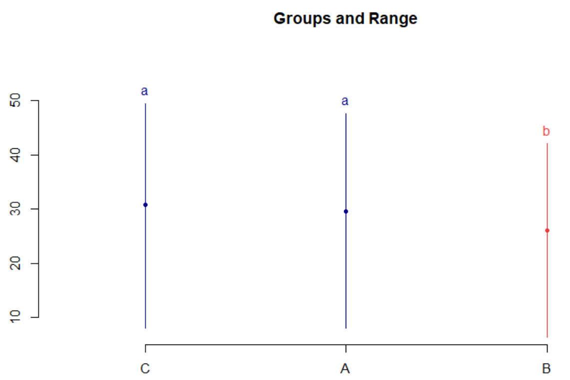

Figure 14 Range values of maximun temepratures at each growing location. Groups represents each location after running Tukey HSD test with 0.05 significance maximum values of temperature.

The profile of temperatures at each location during the summer season, starting from February and finishing at the end of July when the floricane harvest was ended. The different managements can be distinguished by observing the earliness of reaching high temperatures at the environment C and the late scheme from location B maintaining low temperatures longer during the cycle. It can be also observed the temperature range between minimum and maximum is very close in the environment B mainly during the harvest period. Location C shows a marked change of temperatures, increasing considerably the maximum values after budbreak (Figure 15).

Figure 15 Maximum, minimum and average temperature values registered at the location A, B and C.

To evaluate the influence of the temperature on the phenology of the plants, growing degree hours were calculated for the vegetative period. After running ANOVA test, no significant differences were found, which means, that even if there were differences between registered temperatures, no significant differences were found on its influence on the growing cycle of the plants between the three locations and a significant level of 95%.

4.3. Phenology

Table 4 presents the results obtained from the data collected regarding phenology statements of the plants, including the date, the week number, the GDH cumulated till that date and the length of each period. The complete period of budbreak for the three growing sites was completed after the week number 13, which corresponds to the first week of April. No statistical differences were observed between locations, or selections regarding the budbreak. As a descriptive analysis, can be described that the budding period was different between genotypes. AMO-022 was more homogeneous and concentrated, in comparison to the other two selections. AMO-008 and AMO-013 presented a larger number of days till completing the opening of buds. This effect can be considered as an influence of the plant material, considering it is an over winter cane (Figure 16).

Table 4 Phenology dates for each genotype at each location.

SELECTION	LOCATION	80% budbreak	week	5% Flowering	GDH	week	Length flowering (days)	Beginning Harvest	GDH	week	Length Harvest (days)
AMO-013	A	4/1/2022	13	4/15/2022	9268	15.	35	5/16/2022	16088	20	33
AMO-013	B	3/25/2022	12	4/22/2022	8842	16	35	5/27/2022	16822	21	23
AMO-013	C	3/4/2022	9	4/1/2022	8198	13	49	5/13/2022	16180	19	38
AMO-008	A	4/1/2022	13	4/15/2022	9268	15	35	5/18/2022	16629	20	31
AMO-008	B	3/25/2022	12	4/22/2022	8842	16	35	5/27/2022	16822	21	27
AMO-008	C	3/11/2022	10	4/8/2022	8488	14	42	5/17/2022	16025	20	34
AMO-022	A	3/25/2022	12	4/22/2022	10591	16	35	5/20/2022	18047	20	32
AMO-022	B	4/1/2022	13	4/29/2022	10253	27	35	5/30/2022	18903	22	26
AMO-022	C	3/18/2022	11	4/8/2022	10488	14	42	5/23/2022	18542	21	39

The early selection, in terms of flowering was AMO-013. The three selections had a stable behavior in between the three locations. The phenology state started after two weeks after the sprouting following the same timeline in between selections and locations. AMO-013 behave as an early cultivar, and AMO-022 as a late one. During budbreak, AMO-013 presented a phenomenon of early flowering, which means that before developing vegetative laterals, flower structures were developed (Figure 16).

Harvest started 5 weeks after the first open flowers. At location A, fruits of the three selections started to be picked at the week 20. At location B, harvest started one week later for AMO-013 and AMO-008 and two weeks later with AMO-022. The third growing field (location C) started the harvest one week early than location A with AMO-013(week 19), week 20 stated the picking of the benchmark and one week later (week 21) we tattered picking fruits from AMO-022.

The profile of bud burst period, observed on Figure 17, differentiates the three genotypes and AMO-022 has the closest and most similar lines on the graph, while AMO-013 hast different curves according to the environment, showing a higher response to the temperatures at location C for the early beginning of the cycle.

Regarding the length of the flowering period Location C presented longer days for the three cultivars. Blooming lasted between 35 and 49 days, and harvest had a length of between 30 and 39 days from the first till the last pickings. Figure 17 illustrates the progress of budding and flowering for the three locations, comparing the three locations. One interesting fact is to observe is that AMO-013 started blooming when bud burst period still was not completed. Flowering line graph shows the behavior of each genotype. AMO-008 and AMO-022 responded with higher number of open flowers to the high temperature management of environment C.

On supplement 2 it can be observed the profile of the harvest period of the three genotypes at each environment. Selection AMO-022 showed the closer lines on the graphs, indicating the stability of the genotype across the three growing fields. AMO-008 and AMO-013 had a considerable later harvest period on location B, responding to the tunnel management for later production.

Figure 16 Phenology stages observed on the field. A: open bud was considered as the unfolding of the first leaves, B: open flower observed on the plants while counting the blooming period, C and D: early flower buds developed before vegetative growth observed on AMO-013.

Table 3 includes growing degree hours required for flowering and maturity. AMO-013 had the same requirements as AMO-008, between 8000 and 9000 GDH till flowering and 16000 hours till harvest. AMO-022 needed over 10000 GDH till flowering and over 18000 GDH till harvest. The cumulation of growing degree hours at each environment represents the tunnel management influence on the temperature.

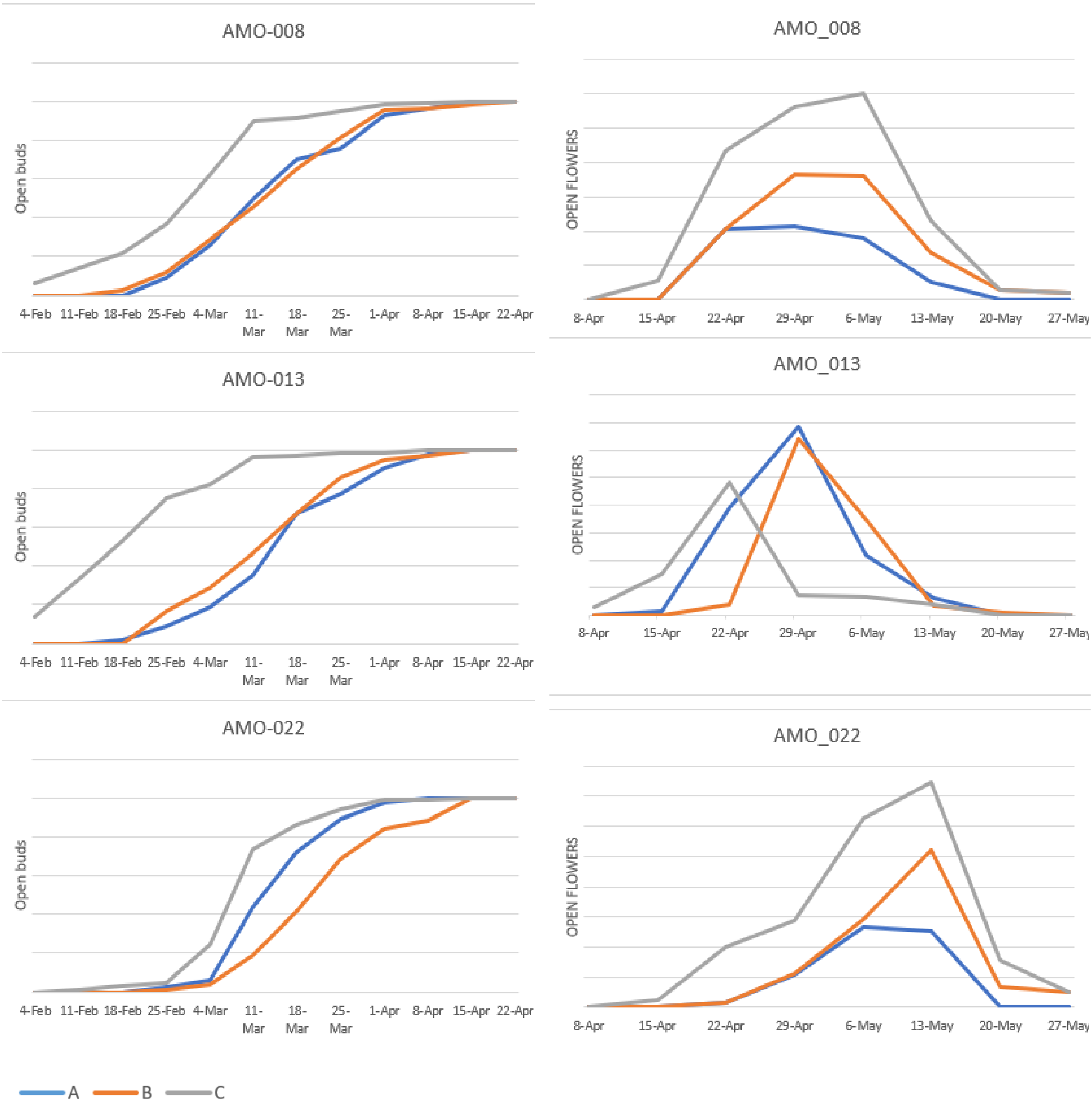

Figure 17 Budding and blooming periods. Left: Bud burst period for each genotype at each growing site. Right: flowering period for each genotype at each location. Vertical axis represents percentage of open buds and open flowers respectively. Blue line: location A, Orange line : location B, Grey line: location C.

4.4. Yield components

Winter cane was characterized by measuring diameter, internode, and number of buds. High environment influence was detected. Cane diameter was significant different between selections, locations, and the interaction of both factors. Number if buds have a high influence of location, but this should be considered as a result of the pruning management of each grower. Percentage of open buds was defined by the genotype, and it is the only trait with no significant differences by the location or the interaction between factors. Number of fruits per lateral, lateral length and berry weigh are statistically different between selections, location and an interaction between factors is observed on the results (Table 5).

*Table 5 Statistical significance of treatment effects from ANOVA of winter cane traits and yield components. NS, *, **, ***Nonsignificant or significance p < 0.05, 0.01, or 0.001, respectively.*

TRAIT	SELECTION	LOCATION	SELECTION: LOCATION
Cane diameter	**	***	*
Internode length	NS	***	NS
Number of buds	*	***	*
Percentage of budding	***	NS	NS
Laterals per cane	***	***	**
Lateral length	*	*	***
Berries per lateral	***	***	***
Berry weight	***	***	***

4.5. Fruit production

Production was calculated considering kilograms of fruits per pot, considering the number of buds that were counted at the beginning of the vegetative cycle. Table 6 shows the different values obtained for yield, considering expected yield, calculated from the measurements done on the different fruiting structures of the plant, the real yield obtained from the sum of the weight of all the harvested berries and another option for calculating potential or expected yield from yield components. Comparing the two selections with the benchmark, AMO_022 showed the best performance, having similar percentage of first quality fruits, while AMO-013 did not show the capability of producing competitive fruits considering fresh fruit quality.

Evaluating potential yield, based on the number of buds per cane, the grams harvested per bud, showed meaningful difference (alpha 0.05) between locations, being location C the one with higher potential yield, for the three selections (Figure 18). Statistically differences were not found in between selections and locations considering kilograms of fruit per pot.

Table 6 Yield values obtained from each genotype at each location, including percentages of first and second quality harvest. Different letters indicate significant difference, NS Nonsignificant or significance difference (P ≤ 0.05).

SELECTION	LOCATION	EXPECTED YIELD (kg/pot)	REAL YIELD (kg/pot)	EXPECTED YIELD (g/bud)	First quality %	Second Quality %
AMO-013	A	1.54 NS	0.75 NS	1.83 a	46.89 b	53.11 b
AMO-013	B	2.67 NS	0.56 NS	1.31 a	29.00 b	71.00 b
AMO-013	C	1.26 NS	0.64 NS	4.71 b	31.77 b	68.23 b
AMO_008	A	1.22 NS	1.00 NS	2.88 a	74.46 a	25.54 a
AMO_008	B	1.15 NS	0.81 NS	2.28 a	80.23 a	19.77 a
AMO_008	C	1.43 NS	0.81 NS	4.74 b	64.08 a	35.92 a
AMO_022	A	1.44 NS	0.91 NS	2.56 a	73.60 a	26.40 a
AMO_022	B	1.15 NS	0.67 NS	1.58 a	77.20 a	22.80 a
AMO_022	C	1.12 NS	0.76 NS	3.98 b	70.84 a	29.16 a

Harvest quality resulted significantly different for AMO-013, from which the lowest percentage of first quality fruit was harvested (Figure 19). A percentage of 35% was the average first quality berries picked for this genotype, presenting the same behavior under the three growing fields (Figure 20). Main defects observed in between the second quality fruits were wrinkled berries, white drupelets, small size, irregular shape, and double fruits (Figure 21).

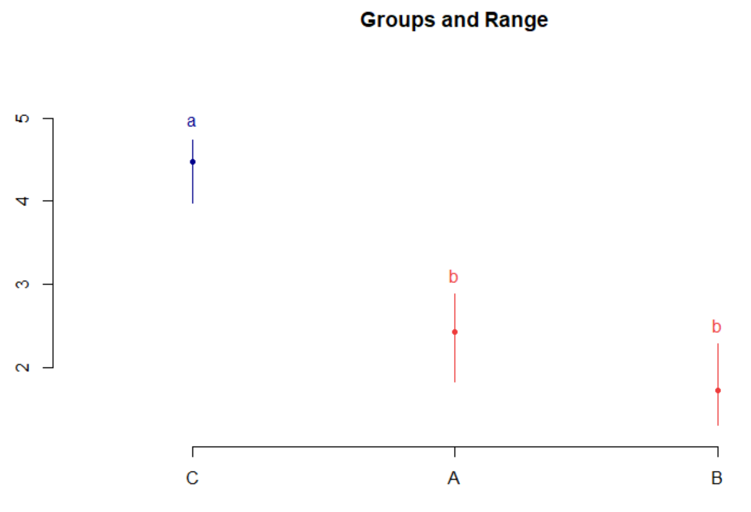

Figure 18 Groups based on potential yield per bud at each location, considering 95% of confidence level. The same letter in the same row indicates no significant difference (P ≤ 0.05).

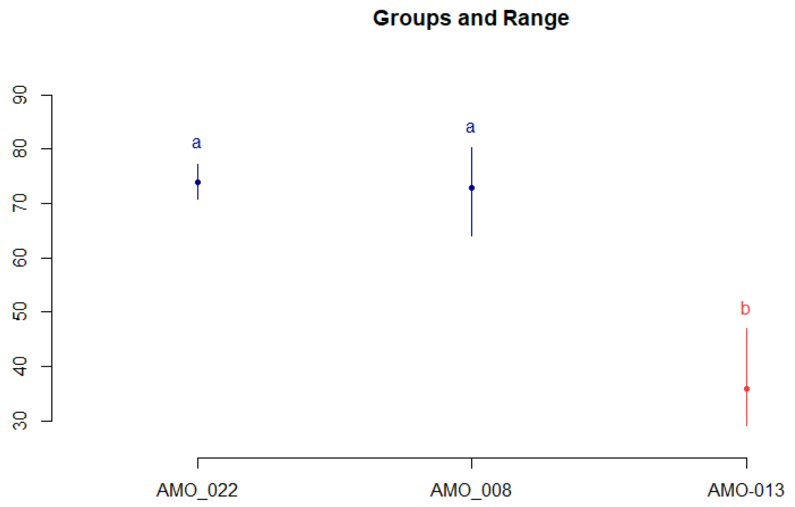

Figure 19 Groups by percentage of first quality percentage of harvest. The same letter in the same row indicates no significant difference (P ≤ 0.05).

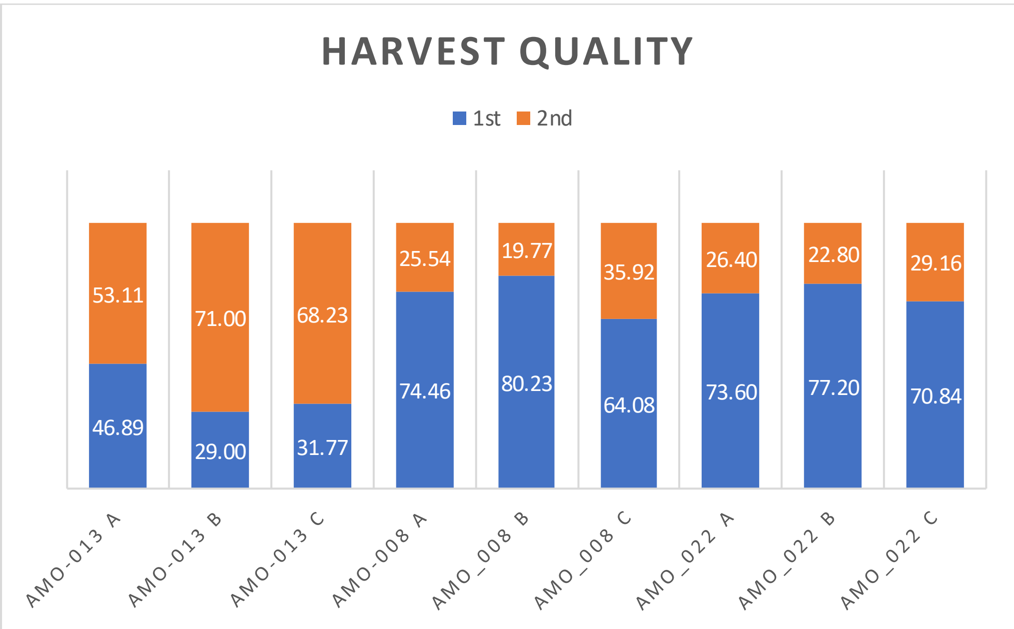

Figure 20 Percentage of first and second quality harvest at each location for each genotype. Each bar corresponds to one selection at one location. Blue section of the bar represents first quality harvest and orange area includes second quality percentage of the total harvest.

Figure 21 Second quality fruits obtained from AMO-013 at the three growing fields. Main defects observed: double fruit, crumbling berries, small size.

4.6. Fruit quality

4.6.1. Physicochemical evaluation

Significant differences were found between fruit weight in between the three growing environments. Location C presented higher values of fruit weight and location B the lowest. This trait is one of the most influenced traits by the environment (Figure 22).

Between the three genotypes, AMO-022 was statistically diffetent (Figure 22) from the other two selections, having a higher weigh mean and arriving to a higher maximun weight of 11 grams. AMO-008 and AMO-013 presented a mean weight value of 4.62 grams and 5.53 grams respectively while AMO-022 presented a mean value of 7.01 grams per fruit (Table 8).

Table 7 summarize the main effects of the factors on the fruit's attributes measured on this study. Fruit width and titratable acidity were the traits with no influence from the genotype or the environment.

*Table 7 Statistical significance of treatment effects from ANOVA of fruit traits. NS, *, **, ***Nonsignificant or significance p < 0.05, 0.01, or 0.001, respectively.*

TRAIT	SELECTION	LOCATION	SELECTION: LOCATION
Fruit Length	***	***	***
Fruit Width	NS	NS	NS
Fruit Weight	***	***	***
Soluble Solids	***	***	*
Titratable Acidity	NS	NS	NS
Ratio SS/TA	*	***	NS

Table 8 Mean value ± standard deviation (x ± SD) of the main attributes measured on the berries for size and chemical composition.

	FRUIT LENGHT		FRUIT WIDHT		FRUTI WEIGHT		SOLUBILE SOLIDS		TITRABLE ACIDITY		SS/TA	
AMO_008	23.41	±2.14	23.48	±19.29	4.62	±0.70	8.84	±0.94	1.56	±0.18	5.79	±1.24
A	21.80	±1.92	28.17	±33.23	4.73	±0.78	9.68	±0.61	1.45	±0.20	6.84	±1.44
B	24.29	±1.89	20.46	±1.15	4.53	±0.57	8.47	±0.85	1.63	±0.13	5.24	±0.80
C	24.15	±1.61	21.82	±1.27	4.60	±0.72	8.38	±0.74	1.60	±0.17	5.28	±0.60
AMO_022	26.86	±4.70	25.52	±2.68	7.01	±1.83	8.69	±1.40	1.54	±0.18	5.72	±1.21
A	23.12	±3.23	24.92	±2.79	6.17	±1.18	9.57	±1.30	1.59	±0.20	6.14	±1.26
B	26.60	±2.11	24.52	±1.87	6.13	±0.82	7.43	±0.89	1.53	±0.21	4.97	±1.01
C	30.85	±4.61	27.12	±2.59	8.73	±1.93	9.08	±1.02	1.51	±0.12	6.07	±1.03
AMO-013	23.57	±3.69	22.48	±2.20	5.53	±1.31	7.91	±0.92	1.57	±0.22	5.18	±1.06
A	20.03	±1.73	22.09	±2.61	4.60	±0.67	8.58	±0.83	1.48	±0.31	5.99	±1.16
B	24.34	±3.00	21.51	±1.59	5.47	±1.07	7.30	±0.69	1.61	±0.16	4.59	±0.67
C	26.34	±2.85	23.84	±1.59	6.53	±1.31	7.87	±0.81	1.61	±0.15	4.95	±0.79

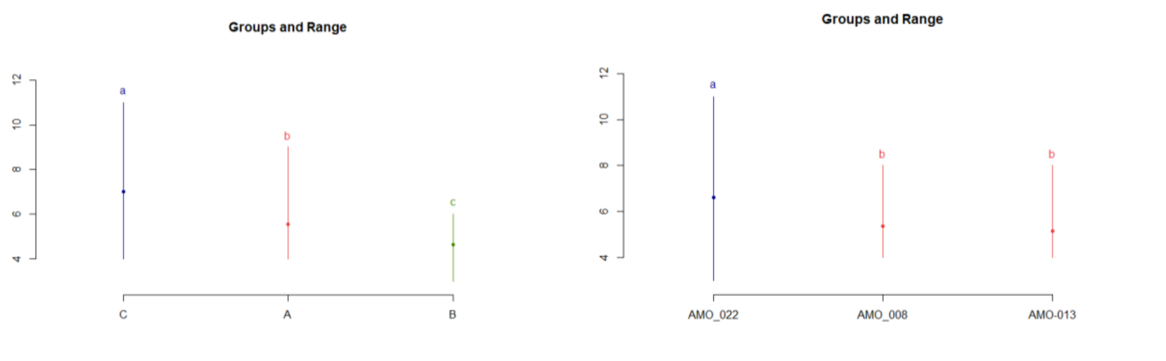

Figure 22 Fruit weight(grams) by location (left), and by selection (right). Different letters indicate significant difference (P ≤ 0.05).

Concerning content of solubile solids and percetnage of acidity, no significant differences were found in terms of acidity. Brix content (Figure 23), and sugar/acid balance (Figure 24) were significant different in between selections and locations.

The selection AMO-013 presented the lowest content of soluble solids, and lower ratio (SS:TA), while AMO-022 and AMO-008 presented values closer to 10 which is an optimin value for raspberries, considering a balanced taste between the sweetness and acidity.

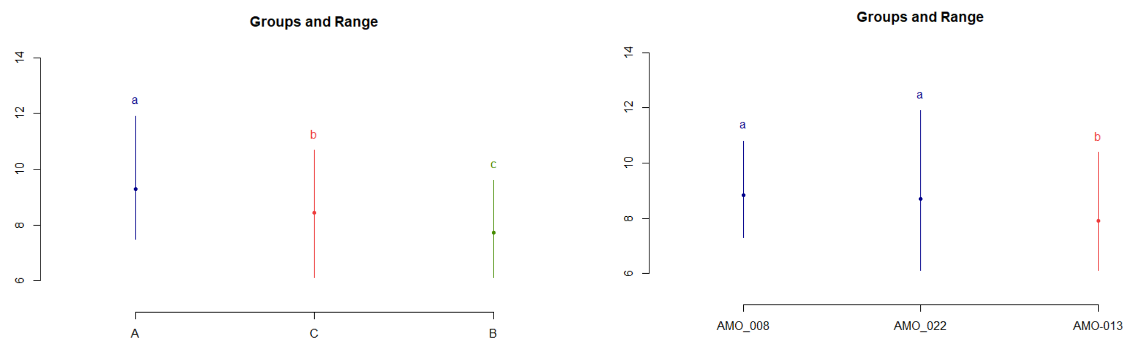

Figure 23 Different groups according to Brix content by location and selection. Same letter indicates no significant difference ($P \leq 0.05$).

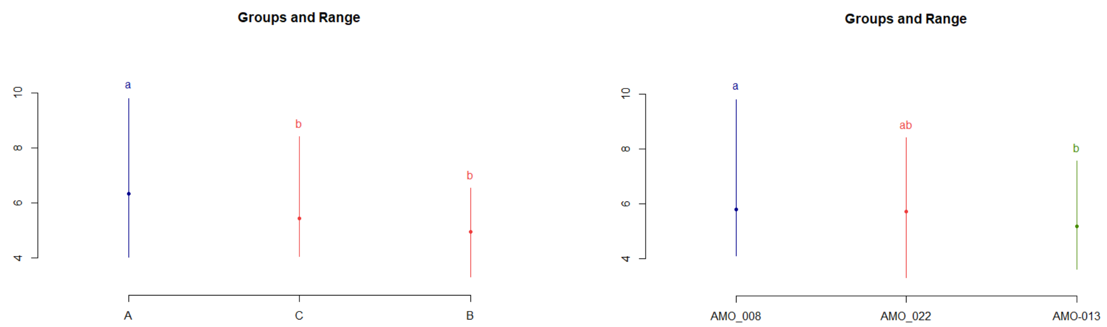

Figure 24 Ratio SS/TA by location and selection. Same letter indicates no significant difference ($P \leq 0.05$).

4.6.2. Shelf life

4.6.2.1. Visual aspect

General aspect of the fruits decrease as the post-harvest days were increasing, color got darker (Figure 25) and firmness decrease. Main quality losses were noted from day 7 and at day number 10 quality was very low and fungal development was observed on some fruits (Table 9).

Table 9 Significant differences between samples during cold storage under the effect of the number of post-harvest days. Same group letter indicates no significant difference ($P \leq 0.05$).

GROUPS	DAY	AMO_008	AMO_013	AMO_022
a	0	5.0	5.0	5.0
a	4	4.9	4.2	4.9
b	7	4.1	2.7	3.7
c	10	3.1	1.3	3.5

Figure 25 Change of color during cold storage. Left picture corresponds to day 0 and right picture corresponds to day 7 for the same sample from the selection AMO-022.

Main factor with significant effect on the post-harvest quality of the fruits was the genotype. In between selections statistical differences were found comparing AMO-013 with AMO-022, and AMO-013 with AMO-008 (Figure 26). AMO-013 was qualify with the lowest values after harvest mainly showing dark color.

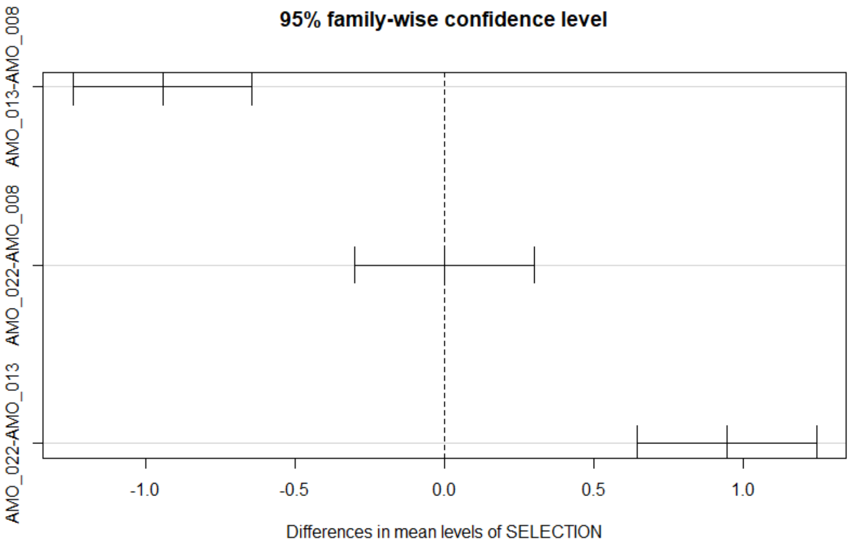

Figure 26 Differences between selections at day 7 during cold storage based on change of color and general aspect of the fruits. Confidence intervals containing zero indicate that there is no difference ($P \leq 0.05$).

4.6.2.2. Weight loss

Fruits harvested at location C was significantly different and presented the higher values of weight loss during the shelf-life test after 7 days in cold storage. At 4 and 10 days in cold storage no significant differences between samples (Figure 27).

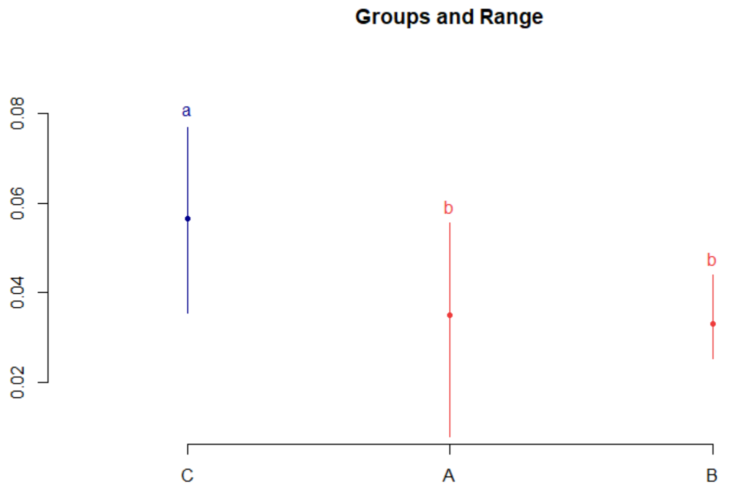

Figure 27 Groups of weight loss by location. The same letter in the same row indicates no significant difference (P ≤ 0.05).

4.6.2.3. LEAKAGE

Leakage distinguished the selection AMO-013, with a mean of 10.83%, as the one with higher values and also having a wider range of values, from 0% up to 50% in between repetitions. AMO-008 and AMO-022 performed similar behavior with a range of percentage of leakage between 0 and 20%. No statistical differences were found on leakage considering location as a factor (Figure 28).

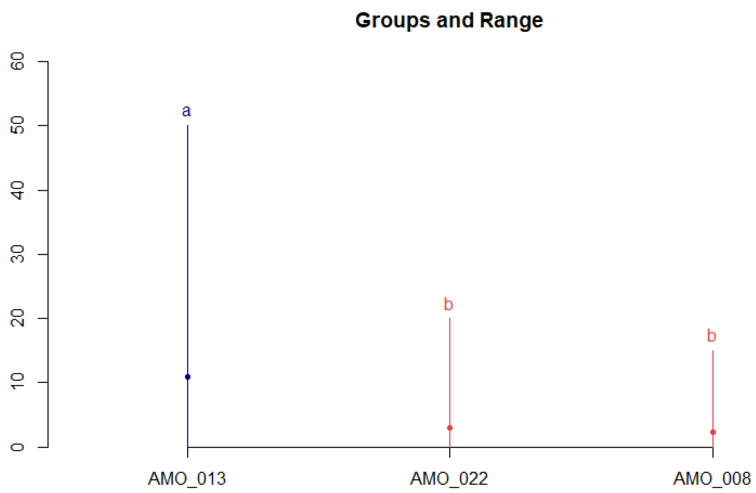

Figure 28 Groups of percentage of leakage by selection at day 7. The same letter in the same row indicates no significant difference (P ≤ 0.05).

4.6.3. Sensory evaluation

Sensory evaluation of the fruits from the three genotypes and from the three growing sites was done and analyzed running an ANOVA test considering the effects of each factor and the interaction. The attributes Color, Shape, Taste, Sweetness and Acidity resulted significantly different between selections. Uniformity, Glossiness and Juiciness presented statistical differences considering the different locations (Table 10).

Liking was also evaluated and divided in visual liking and taste liking. While taste liking presented significant differences between selections, Visual liking was statistically significant between locations.

*Table 10 Statistical significance of treatment effects from ANOVA of sensory attribute responses. NS, *, **, ***Nonsignificant or significance p < 0.05, 0.01, or 0.001, respectively.*

ATTRIBUTE	SELECTION	LOCATION	SELECTION: LOCATION
Color	**	NS	NS
Uniformity	NS	*	NS
Shape	***	**	**
Glossiness	NS	*	NS
Taste	*	NS	*
Firmness	NS	NS	*
Juiciness	NS	**	NS
Sweetness	***	NS	NS
Acidity	***	NS	NS

Sensory profile was built (Figure 29) for the three genotypes based on the responses of the different panel tests carried out during the harvest period. AMO-013 is characterized by high acidity, low values of uniformity and taste. AMO-022 is distinguishable for the level perceived of sweetness, good taste, and uniform berries. While AMO-022 showed good values for all the positive traits of a commercial raspberry, AMO-013 was defined by attributes that are not in correspondence with a good quality fruit.

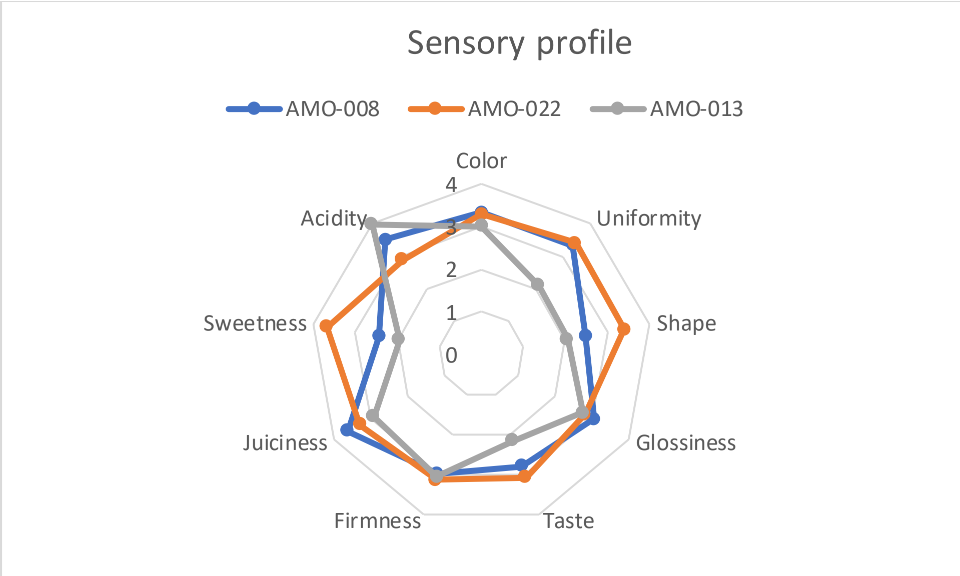

Figure 29 Sensory profile of the three genotypes under study. Orange line describes the parameters for AMO-022, blue line for AMO-008 and grey line for AMO-013.

Principal components analysis was done for the different terms used to describe the fruits during the panel test. The PCA score plot of the first two PCs of a data set about sensory evaluation of the raspberries. This provides a map of how the terms relate to each other. The first component explains 40.75% of the variation, and the second component 12.76% (Figure 30).

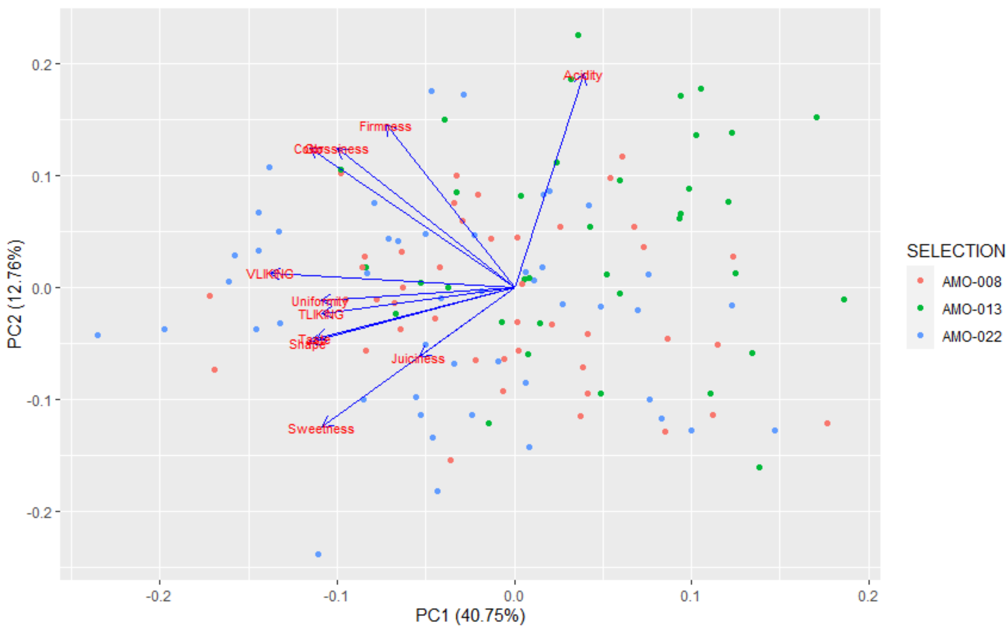

Figure 30 Plot of PCA analysis for the sensory evaluation of the fruits of AMO-008, AMO-013 and AMO-022.

Figure 29 displays the relationships between all sensory variables at the same time. Variables contributing similar information are grouped together, that is, they are correlated. Visual liking, Uniformity and shape are examples of variables that are positively correlated. As well, Firmness, Color and Glossiness are terms closely correlated. In the case of Acidity and sweetness the relation shows an inverse correlation, meaning that when acidity increases, sweetener decreases, and vice versa. As well, Sweetness, taste and taste liking are positively related.

Considering the distance to the origin, the variables Acidity and sweetness are the sensory traits with stronger impact on the model and Juiciness, which is near the center of the plot origin, are not having an important influence to differentiate the samples. Overall, no big differences are shown on the influence of the visual parameters on the model.

Regarding how these factors are related to the two evaluated selections, it can be extrapolated that AMO-022 data, in relation with the taste and sweetness of the fruit and liking, while the data from AMO-013 is associated with the level of acidity.

Deeper assessment was done performing further panel tests including more concepts related with final consumer impressions (CATA questions). Also, post-harvest day as a was included as a factor to compare the sensory attributes after 5 days of harvest. This assessment was only carried out for the selection AMO-008 and AMO-022 for the reason that there were not enough berries of good quality to complete samples for the CATA test.

In between the frequencies of responses for the terms of the CATA test, the answer for very sour has been the one presenting higher difference in between the samples. AMO-008 had an average frequency of 6.25 while for AMO-022 the value was 2. This indicates that this is a characteristic that highly influence the perception of the consumer and that it is not high in the new selection.

Considering the frequencies of the responses from the CATA questions, for each descriptive term radar graph was built for the new genotype that shown good sensory results. The main attributes to describe the selection are, size, shape, sweetness, and raspberry flavor (Figure 31).

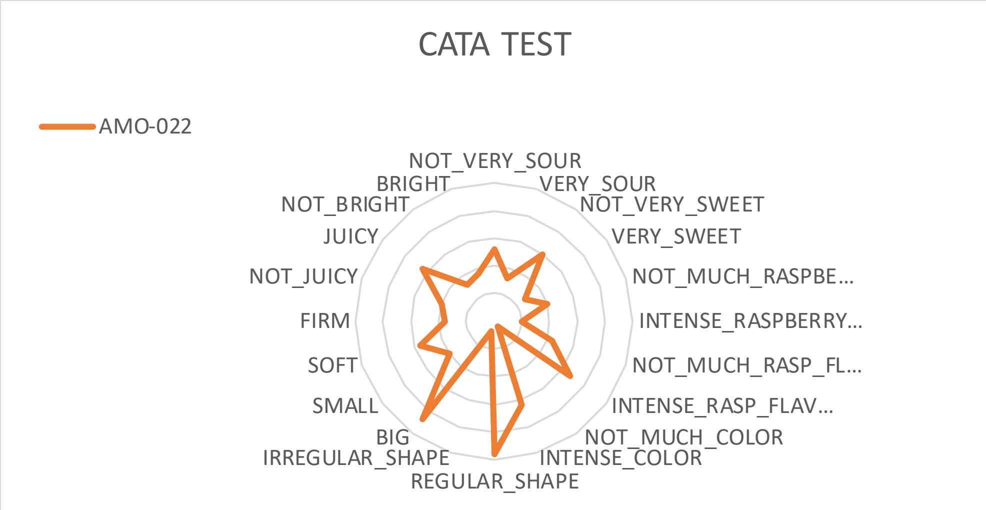

Figure 31 Radar chart for the sensory profile perceived by consumer, built with the responses of the CATA questions for the selection AMO-022.

5. DISCUSSION

As described by Hanson et al. (2019) breeding efforts in raspberries have been focused on the development of primocane-fruiting cultivars and improvements on fruit size and quality. Primocane-fruiting plants are the main trait of new cultivars, considering the advantages that this fruiting cycle gives to the growers. However, it is essential to know the performance during the floricane production to define the best fruiting cycle of the cultivar.

The purpose of this study was to analyze the performance of two advanced selections and one commercial cultivar in three fields with different locations in the region Emili-Romagna, in particular during the summer production. The main factors considered in this assessment have been the genotype and the agronomic management of the plants and the tunnel. The two advanced selections have been selected as primocane fruiting potential cultivars, with high quality fruit production. The evaluation of the summer production on the floricane is part of the selection process, to observe the potential for double cropping.

The evaluation of the plant material was carried out following UPOV protocols for phenotypic evaluations. The aim of this phenotypic assessment was to describe each genotype and to assure uniformity, stability, and distinguishable characteristics of each cultivar. Main differences are related to the presence of thorns and the shape of the fruit. Since the floricane production is the one obtained from the one-year-old cane, after having enough chilling hours during winter, the plant material determines the final harvest. As the advanced selections are obtained from clonal reproduction, during the vegetative and production cycle, plants were observed and described following standardized morphological characteristics and also to assure the homogeneity of the plant material used on the evaluation.

Environmental conditions did not differ between the three locations in terms of growing degree hours, but they did differ in terms of extremes values of temperature. It can be assumed that the influence of these particular extreme values and the moment when they did occur, had an influence in the quality of harvested berries and the length of the harvest period. Location B, that presented the latest harvest, had a shorter period and lower quality levels, due to the concentration of the production during the warmest period, in which high temperatures and low temperature amplitude affected the maturation of the raspberries. As reported by Nestby et al. (2012), high temperatures after flowering and during berry maturation can affect the quality, producing smaller fruits also reducing the potential yield. Location A and C started earlier with the production, having longer period of harvest, and moving the peak of production before to the period of high temperatures. This aspect, is to be considered as an effect of the agronomic management, considering that the three genotypes had the same response to this climatic condition. At location B, high temperatures affected the maturation of the berries, and concentrated it on a shorter period, and the real yield, was considerably reduced in comparison to the expected one.

Growing degree hours were a useful tool to describe the requirements to flowering and to harvest. This tool allows to characterize cultivars and compare them with a standardized unit of measurement. In agriculture where systems are primarily temperature-driven, a measure of heat unit accumulation accounts for seasonal differences in development time and as described by Black et al. (2008) this is particularly important in managing greenhouses and high tunnels designed to advance fruiting. Single year data is not enough to have certain and final results, but during this study the statistical model was on validation.

Potential yield and real did not show equal results. Final quantity of harvested berries is highly influenced by the plant structure and its ability to hang them till maturity. Loss of production due to fails on the hanging system of the plant, gives reasons to include vegetative traits to be evaluated and to define the correct yield components for decision making when it comes to selection in a breeding program. Considering the results main yield components to be considered are number of fruiting laterals, length, and flexibility of the lateral, to avoid broken ones and to assure their ability to reach the wires and be able to hold the berries till harvest. As reported by Nestby et al. (2012) fruit quality and marketable production is highly influenced by the architecture of the plant, in this study one of the selections, AMO-013 did not produce good quality berries neither a good quantity due to broken laterals during fruit maturity. Laterals did not reach the wires and they did not support the weight.

Considering the assessment has been done only on the floricane season, and the plant material is a one-year-old cane, previous year's conditions should be taken into consideration to have reliable results. It was confirmed by the measurements done on the winter cane. Diameter, and internode length were highly influenced by the location, and it is the result from last season growth, and that might affect the development of the fruiting laterals. There are some authors as Palonen et al. (2021) that have studied the behavior of different cultivars during floricane production and primocane production, trying to identify the best fruiting cycle, considering not all raspberry cultivars are suitable for double cropping. According to the productive results of this assessment, one of the selections did not perform well in terms of vegetative grow and production. The other one, AMO-022, had a competitive performance in comparison with the benchmark, with even better results in terms of yield and berry quality.

Division between first and second quality harvest maintained the same proportions across the three growing sites and it differences were significant in between genotypes. The differences observed give another insight to classify a cultivar as suitable or not for the production cycle.

According to Hanson et al. (2019) the suitable cultivars for double cropping as the ones with high weighed fruits, considering this trait as the main responsible of potential yield. AMO-022 presented the best weighed raspberries, and also best yield. Nevertheless, fruit weigh was significant different between selections, it was also influenced by the environment, being reduced by extreme high temperatures supporting the theory previously reported (Nestby et al., 2012).

The final decision for keeping a raspberry selection for the fresh market, is defined by the quality of the berry. The three locations did show differences for weigh, length, and content of soluble solids. In accordance with Morel et al. (1999), this may indicate maturity differences among the sites due to different picking criteria as well as the influence of the temperature during maturity, considering high temperatures induce higher levels of water loss and reduce growth rate (Nestby et al., 2012).

Although it is known that yield, concentrations of sugars and organic acids, and the sugar to acid ratio in raspberries is affected by growing conditions, there is reported and observed in this report that there is a high genetic influence on how the plant is affected by environmental conditions.

Shelf life, also cited by Contreras et al. (2021), is highly connected to the harvested stage of the berries and also influenced by the genotype. The cultivars that allow to be harvested less mature had a longer shelf life and were firmer after 7 days. Therefore, AMO-022 like the cultivar AMO-008, can be harvested between pink and bright red and ensure better postharvest behavior. The selection AMO-013, turned into a darker color and the general aspect decrease the quality of the berry. Additionally, leakage, presented significant differences for this last selection, having high percentages at day 7 of cold storage, resulting in berries that are not in a level of marketable fresh raspberries.

The application of panel test evaluations of the berries gives important and useful information about the sensory perception of the fruit and give tools for predicting the acceptance from the fresh fruit market. The methods applied on this study are the descriptive test, with the objective of describing the profile of the fruits by giving a mark from a stablished scale to different attributes. Sweetness and acidity resulted to be the most meaning full traits for differentiating cultivars. AMO-022 presented the highest levels of sweetness and acceptability of the panelists.

Returning to the influence of the temperature on the development of the fruits, uniformity, shape, and glossiness were statistically qualify different in between growing sites. Which can be a result as well of the picking criteria and the influence of temperature at the moment of harvest.

PCA allowed to observe the influences of the different traits on the differentiation of the three selections and related to the liking scores obtained from a consumer point of view. Liking is correlated to sweetness and good berry appearance.

The last technique used for fruit evaluation, based on Lado et al. (2010), was the consumer test, by using check all that applies questions. There were not enough good quality fruits of the two selections, and so it was only performed with the benchmark and the most promising selection. Results show a similar trend to the results from the panel test. The selection AMO-022 was qualify as intense raspberry flavor, big size and uniform shape, which are considered as good quality parameters for the fresh market (Palonen et al., 2021).

6. CONCLUSION

The protocol used for this work has been useful to identify main characteristics of the selections and it is simple to be applied in further phenotypic descriptions. A standardized unit to quantify yield still needs to be defined to obtain equivalent results.

Taking into consideration the comparisons done with the collected data, clear differences were observed between the three genotypes under evaluation. The selection with higher potential yield, was the one presenting higher number fruits per plant and with higher weight per fruit. This is considered as the main yield component. The structure of the plant and its capability to hold the production has define the final yield. Not all the plants are capable of holding the berries and laterals fall with the berries before maturity. This plant trait has been influenced by the genotype.

GxE effects were significant mainly on the vegetative structure, considering that the plant material was coming from a previous production. Differences among the sites were small and in general not significant for most attributes related to fruit quality and sensory traits although differences were detected for the uniformity, shape, glossiness, and juiciness attributes.

Yield was evaluated as potential yield, considering the main yield components, measuring them, and calculating the potential yield per bud, which would represent the kilograms per productive lateral, considering homogeneous laterals along the cane. Also yield was calculated as kilograms harvested per plant, which is the real production of the harvest season. The potential yield showed high differences with the real one, so it cannot be considered as a useful tool to predict production, since it is highly influenced by the vegetative development of the plants.

Quality has been observed as the main parameter to distinguish between cultivars. Percentage of first quality harvest, fruit weigh and soluble solids allowed to distinguish and characterize the three cultivars. It is crucial to include sensory evaluation from different moments of the harvest to have a complete overview of the stability of the berries during the harvest period. Check all that applies questions presented a useful tool to identify the main attributes perceived by the consumers.

The genotype AMO-013 did not show a good performance, even though it has been selected as a good material based on the primocane production, it started the flowering before having a vegetative structure, which lead to weak plants when the fruits were ripening, lot of laterals were broken falling with berries before maturity and the ones that could be harvested presented bad quality fruits, with lot physiological disorders on the harvested berries.

The selection coded as AMO-022 appeared to be the best suited for the conditions and managements used in this study. The plant material presented higher yield and presented higher quality berries in comparison with the benchmark. With the characteristics of having conical fruits, a range weight between 6 and 11 grams, good parameters for shelf life and optimal sensory profile. Considering that the previous year the plants produced on the primocanes, this genotype can be considered proper for double cropping.

Based on the results presented here, we can conclude that growing primocane raspberry cultivars as long cane plants for summer cropping (floricane production) is an option that cannot be adapted to all cultivars. Competitive floricane yield with excellent fruit quality were observed in our study for AMO-022, and low first quality production was obtained from the other selection AMO-013, although both of them were already selected as potential cultivars for primocane production.

This study did not include the influence of year effects. Multiple year data should be included, but if the growing seasons are good, and fruit rots are minimal, the advantage of a second year of data is likely to be minor. This assessment is just one step inside a selection process of diverse years and data should be considered as part of a complete multiyear field trial project for final conclusions.

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8. SUPPLEMENT MATERIAL

SUPPLEMENT 1: Standardized phenotypic description of the cultivars

OBJECTIVE		TRAIT TO OBSERVE	CRITERIA	AMO-022	AMO-013	AMO-008
PHENOLOGY		Time of beginning of budding	3-early, 5-medium, 7-late	5	3	3
		Time of beginning of flowering	3-early, 5-medium, 7-late	7	3	3
		Time of beginning of fruit ripening	3-early, 5-medium, 7-late	5	3	3
		Length of fruiting period	3-short, 5-medium, 7-long	5	5	5
MORPHOLOGY	PLANT	Plant habit	1-upright, 2-semi upright, 3-arching	2	1	1
		Current season shoots	3-few, 5-medium, 7 – many	5	5	7
	APEX	Anthocyanin coloration of apex during rapid growth	1-absent, 9-present	1	9	1
		Intensity of anthocyanin coloration of the apex during rapid growth	3-weak, 5-medium, 7-strong	NA	3	NA
	CURRENT SEASON'S CANE	Bloom	1-absent, 3-weak, 5-medium, 7-strong	1	1	1
		Anthocyanin coloration	1-absent, 3-weak, 5-medium, 7-strong	1	1	1
		Hairiness	1-absent, 3-weak, 5-medium, 7-strong	1	1	1
		vigor of the cane	3-low vigor, 5-medium, 7-vigorous	7	5	7
		Length of internode	3-short, 5-medium, 7-long	5	5	5
	DORMANT CANE	Length of the cane	3-short, 5-medium, 7-long	7	7	7

		Color	1-brownish grey, 2-greyish brown, 3-brown, 4-purplish brown, 5-brownish purple	1	2	3
		Length of the internode	3-short, 5-medium, 7-long	5	3	5
		Length of the lateral shoots	3-short, 5-medium, 7-long	7	3	7
		vigor of the cane	3-low vigor, 5-medium, 7-vigorous	5	5	7
	SPINES	Presence	1-absent, 9-present	9	1	9
		Density	3-sparse, 5-medium, 7-dense	3	NA	5
		Length	3-short, 5-medium, 7-long	3	NA	3
		Color	1-green, 2-brownish green, 3-greenish brown, 4-purplish brown, 5-brownish purple, 7-purple	4	NA	4
	LEAF	Green color of upper side	3-light, 5-medium, 7-dark	7	5	7
		Predominant number of leaflets	1-three, 2-equally three and five, 3- five	2	2	2
		Rugosity	1-very weak, 3-weak, 5-medium, 7-strong, 9-very strong	5	3	5
		Pedicel: number of spines	1-absent, 3-few, 5-medium, 7-many	3	1	3
	FLOWER	Size	3-small, 5-medium, 7-large	5	5	5
		Amount	3-few, 5-medium, 7-high	5	7	7
		Peduncle: spines	1-absent, 9-present	9	1	9
	FRUIT VISUAL DESCRIPTION	Length	3-short, 5-medium, 7-long	7	3	5
		Width	3-narrow, 5-medium, 7-broad	5	7	5

		Ratio length/ width	3-small, 5-medium, 7-large	7	3	5
		Shape in lateral view	1-circular, 2-broad conical, 3-conical, 4-trapezoidal	3	2	3
		Size of single drupe	3-small, 5-medium, 7-large	5	3	5
		Color	1-yellow, 2-orange, 3-light red, 4-medium red, 5-dark red, 6-purple, 7-dark purple	4	5	3
		Glossiness	3-weak, 5-medium, 7-strong	5	7	5
		Firmness	3-soft, 5-medium, 7-firm	7	5	5
		Adherence to plug	3-weak, 5-medium, 7-strong	5	3	3
		Main bearing type	1-only on previous years cane in summer, 2- both on previous year's cane in summer and on current year's cane in autumn, 3- only on current year's cane in autumn	2	2	2
	OBSERVATIONS	Integrity of the drupelets	1-low, 9-high	9	1	9
		White drupelets	1-Presence, 9-Absence	9	1	1
		Uniformity	1-not uniform, 9-uniform	9	1	9

SUPPLEMENT 2: Harvest period for selections at each environment