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Fundamental and Applied Aspects of Plum (*Prunus domestica*) Breeding

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ABSTRACT

The hexaploid European plum (*Prunus domestica* L.) is one of the most important temperate fruit crops. Its origin is unclear as wild forms are missing. The genetic base which can be used for breeding is highly diverse and provides a good base for further improvement of the fruit crop. Information on the inheritance of single traits are rarely available. Breeding focuses on resistance and fruit quality. Classical breeding is the most important method applied. Very few data is available on the genome sequence. No marker assisted selection systems are available. Genetic engineering is limited to the transformation of embryonic tissue derived from seeds. *Prunus domestica* is the only *Prunus* species where genotypes completely resistant to the *Plum pox virus* exist. This resistance is based on a hypersensitive response of the plant cells to the virus. Interspecific hybridization becomes more important in terms of transferring resistance traits from European plum to related species and of developing hybrids with new fruit characters. Classical breeding is far from being the limit of the improvement of plum genotypes.

Keywords: European plum breeding, Sharka resistance, *Plum pox virus*, hypersensitivity resistance, fruit quality
Abbreviations: APFL, amplified fragment-length polymorphism; **DNA**, deoxyribonucleic acid; **ELISA**, enzyme linked immunosorbant assay; **PCR**, polymerase chain reaction; **PPV**, *Plum pox virus*; **RAPD**, randomly amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **SSC**, soluble solids content; **sRNase**, stylar Ribonuclease; **SSR**, single sequence repeat

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ORIGIN AND HISTORY OF THE CROP

Plums are temperate fruit trees with soft fruits. The seed is covered by the lignified endocarp which is called stone. The mesocarp forms the fruit flesh which is encircled by the exocarp, the skin. Fruits of a broad range of different species are called plum: (1) The diploid myrobalane (*Prunus cerasifera*) which is widespread through whole Europe, Asia Minor and Asia. Their fruits are usually soft, sweet and have a sour skin. (2) The tetraploid sloe (*P. spinosa*)

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which is also widespread similar to *P. cerasifera*. It bears small, dark blue fruits (6-16 g) with bitter and adstingend fruit flesh and skin. (3) The diploid Japanese plum which is a conglomerate of *P. salicina* genotypes and of hybrids between *P. salicina* and mostly North American native diploid plum species such as *P. americana*. (4) The diploid native North American plum species such as *P. americana*, *P. nigra* and *P. besseyi* which are not cultivated but contrib.-uted to the development of the Japanese plum type. (5) The hexaploid European plum (*P. domestica*) which is widespread in Europe. This species will be described as follows.

A wild type of *Prunus domestica* (European plum) and especially the typical form of this species, the prune, is unknown. Crane and Lawrence (1934) suggest that the hexaploid *P. domestica* (2n = 6× = 48, genome formula CCSSSS) is an amphidiploid (allopolyploid) hybrid of *P. cerasifera* Ehrh. (cherry plum, diploid, 2n = 2× = 16, CC) and *P. spinosa* L. (sloe, tetraploid, 2n = 4x = 32, SSSS). Rybin (1936) found spontaneous intraspecific hybrids in the Caucasian region. One of this natural hybrids had 2n = 48 chromosomes and was morphological indistinguishable from the common plum. He also crossed *P. spinosa* and *P. cerasifera* and obtained seedlings which were regarded as resynthesised *P. domestica*. Most of these plants were sterile and had no or only some fruits. However, one hybrid was highly fertile; this supported Crane's assumption of the origin of *P. domestica*. A similar experiment was made by Endlich and Murawski (1962). Interpreting cytological studies on interspecific hybrids between *P. cerasifera*, *P. spinosa* and *P. domestica* Salesses and Bonnet (1994) concluded that *P. spinosa* itself is an allopolyploid hybrid between a unknown diploid type of *P. spinosa* (SS) and *P. cerasifera* (C'C'). Considering these findings the genome of *P. domestica* might consist of two *spinosa*- and four *cerasifera*-derived genomes (SSC'C'CC). Considering the observation that the frequency of unreduced gametes is very low in *P. cerasifera*, Eryomin (1991) proposes diploid *P. cerasifera* (CC) × *P. salicina* (SaSa) hybrids which have the tendency to produce unreduced gametes to have contributed to the development of *P. domestica* (SSCCCSa). The hybrid nature of *P. domestica* is nowadays widely accepted and it is assumed that the species originated in the Caucasian region because both *P. cerasifera* and *P. spinosa* are native there. However, there are reasons for considering another origin of the *P. domestica* group. As there are only very few morphological similarities between sloe and European plum, some authors suggest *P. domestica* to be an autopolyploid hybrid of *P. cerasifera* (Beridze and Kvatchadze 1981; Zohary 1992) (CCC'C'C''C''). Up to know, the origin of *P. domestica* remains somehow mysterious. Bullaces and Damsons may be regarded as more primitive forms of the *Prunus domestica* group. As no dark blue genotypes are known in *P. cerasifera*, *P. spinosa* seems somehow to be involved in the evolution of European plu*m.* New approaches should be made using molecular marker techniques to find the origins of *P. domestica*. Cytological studies using the light microscope are very difficult because the chromosomes of *Prunus domestica* and its relatives are very small (about 2 µm).

Plum has been the first fruit species to attract human interest (Faust, and Surányi 1999). As grave-finds show, plums have been known in Europe since 6,000 years (Erémenyi 1977). The plum was known to Greeks and Romans. Romans contributed to their cultivation and spread throughout Europe. In Central Europe, dried *P. domestica* fruits contributed to a large extent to the carbohydrate and vitamin supply of the rural population during winter times. In South Germany, at the end of the 19th century, half of the fruit trees have been plum trees.

The origin and dissemination of plums is reviewed by Faust and Surányi (1999).

BOTANICAL DESCRIPTION AND GENETIC RESOURCES

The taxonomy of European plum within the family *Rosaceae*, the subfamily *Prunoideae* and the genus *Prunus* is controversially discussed since decades. According to Hegi (1906) the European plum is a member of the *Prunophora* subgenus which itself is subdivided into the sections *Prunocerasus* and *Euprunus*. In the section *Prunocerasus*, the North American species bearing small fruits such as *P. americana*, *P. angustifolia*, *P. hortulana*, *P. munsoniana* and *P. maritima*) are subsumed. The European plum group belongs to the *Euprunus* section which contains the plum species present in Europe and Asia, among them *P. cerasifera*, *P. spinosa* and *P. salicina*. Röder (1939) supports the opinion given by Rybin (1936) to see *Prunus domestica* as a general conglomeration of *per se* quite variable cultured forms of different plum races present in Europe. He proposes three subspecies within *P. domestica*: ssp. *insititia* (mirabelles and so called 'Spillinge'), ssp. *oeconomica* (prunes) and ssp. *italica* (plums, reineclaudes and all other kinds of plum fruits). This categorization is only based on fruit characters. Seedlings originating from self pollinated flowers of a *P. domestica* ssp. *insititia* genotype showed high variability and could be allocated to different subspecies (Schmidt 1954). Therefore, Schmidt (1954) and Johannson and Oldén (1962) conclude that a subdivision of *P. domestica* cannot be justified from the genetic point of view. The author's observations support this statement. Molecular techniques such as microsatellite analysis of the chloroplast and nuclear genome are necessary to lighten the taxonomic relationship within the *P. domestica* group. Interspecific hybridization is more successful between *P. domestica* and *P. armenica* than between *P. domestica* and *P. salicina*. This could indicate that *P. armeniaca* might me more close to *P. domestica* than *P. salicina* is. Therefore, the taxonomic relationship between *Prunus* species should be reinvestigated.

Considering the fruit characters, the following main groups can be distinguished. All groups can be hybridized, and intermediate forms between all of them exist. As shown by Werneck and Bertsch (1959), the morphology of the stones can be well used for discriminating between these groups. The stone characters are the best morphological markers for identifying genotypes (Anders 2009). **Fig. 1** gives an example of stones of two different plum cultivars.

Plums

Plum fruits (**Fig. 2A, 2B, 1I**) lose their texture when heated. Frequently, they are round to oval in different sizes and colours. The flesh is juicy, soft and mostly clingstone. Usually, the fruits are ripening earlier than those of prunes but there are many exceptions from this rule. The fruits are mostly used for fresh consumption, compote or canning. The soluble solids content ranges between 12–25% Brix. The titratable acid content is usually lower than that of prunes.

Prunes

During cooking or baking, prunes keep their texture very well and loose less sap than plums. Therefore, they are used for the famous plum cake. Often, the fruits are oval to elongated, usually smaller than plums and generally high in sugar content (up to 30% Brix). They can therefore be well used for drying. The color is mostly dark blue to purple, but there are also some cultivars with red, pink, yellow or bright color (**Fig. 2C-G, j**). They are used for fresh consumption, cooking, baking, drying and distillery. The titratable acid content is usually higher than that of plums. Flesh color ranges from green and yellow to orange. Young shoots of prune trees are not pubescent.

Typical prunes are 'Prune d'Agen' and 'German Prune', which is the most spread prune in Europe, called 'Hauszwetsche' in Germany, 'Pozegača' in Yugoslavia, 'Beszter-

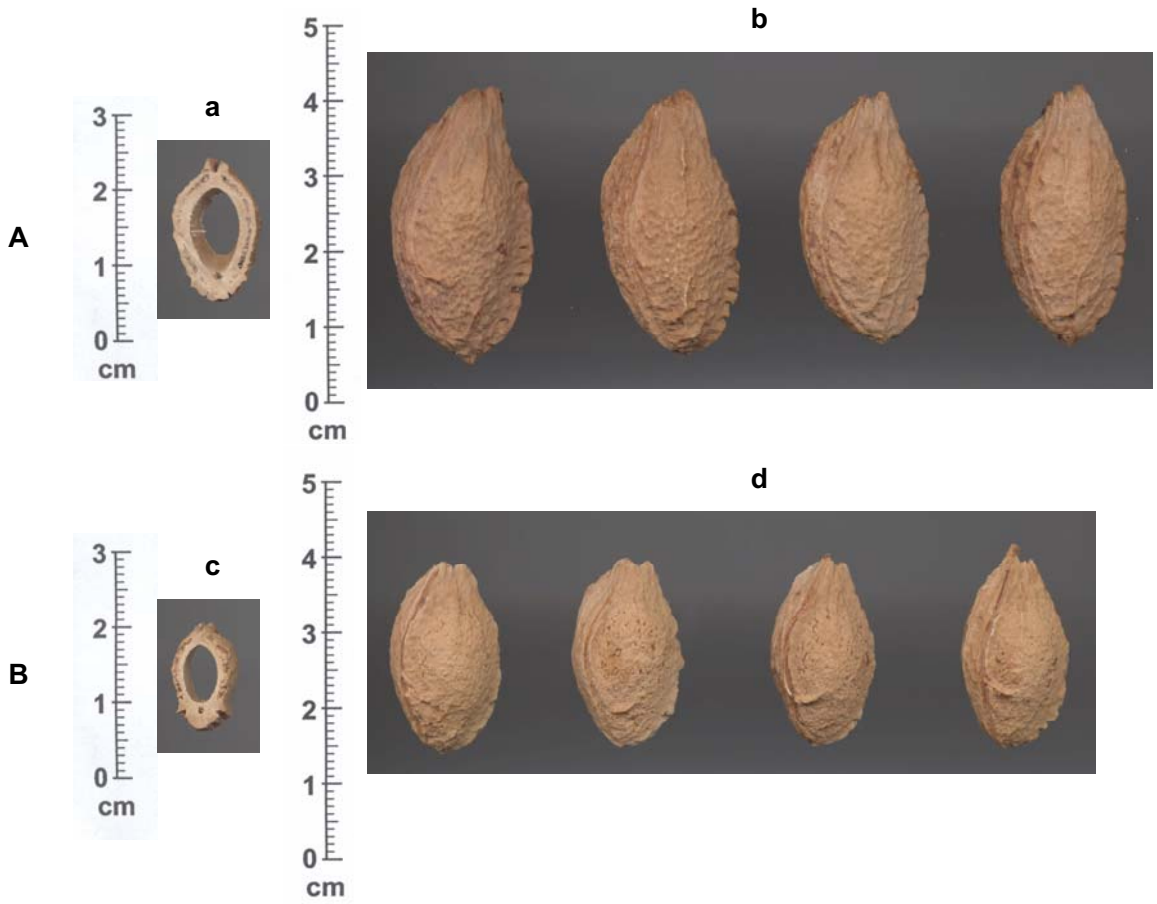

Fig. 1 The stone morphology can be used for the identification of cultivar. (**A, B**) 'Dimbovita'; c, d: 'Carpatin'. a, c: cross sections; b, d: lateral surfaces of the stones.

cei' in Hungary, 'Casalinga' or 'Dro-Zwetsche' in Italy, 'Quetsche Commune' in France, 'Vinete romanesti' in Romania and 'Kustandilska' in Bulgaria, in some countries known as 'Commun Plum'.

Reineclaudes

Reineclaudes (also called gage plums) have round fruits in different colours ranging from green ('Green Gage') to yellow ('Oullins') and purple ('Graf Althans') (**Fig. 2K**). The flesh is juicy, sweet, with aroma, very tasty and of high quality. The fruits are used mostly for fresh consumption, sometimes for brandy production. Reineclauds are highly aromatic plums.

Mirabelles

Mirabelles have small round fruits (8–12 g) with a diameter between 22–28 mm (**Fig. 2L**). They are mostly yellow colored, often with red spots, but there are also green and more purple colored cultivars. The fruit is freestone, juicy, very sweet (18–32% Brix), and full of aroma and of high quality. They are used especially for canning and brandy industry, but in the last time more and more for fresh consumption, too. Most famous are 'Mirabelle de Nancy' for the fresh market and 'Mirabelle de Metz' for brandy production.

Primitive forms and autochthonus biotypes

In many countries, primitive, mostly local forms of *P. domestica* (**Fig. 2H**) have been found. They are grown on their own roots and are propagated by suckers. In former times, they were cultivated, but nowadays they are only found growing in not cultivated hedges or on the skirts of forests. In Middle Europe, Spillinge are well known. They were described by Tabernaemontanus (1588) for the first time. Different autochthonous biotypes were found by Werneck (1958) in Austria. The resistance qualities of local and old plum cultivars and primitive landraces were described by Paunovic (1988). In Hungary, local plum genotypes were used for breeding purposes (Surányi 1998). In Bulgaria, local cultivars were studied by Ivanova *et al.* (2002) and in Romania by Botu *et al.* (2002).

A detailed overview on the genetic resources of plums is given by Ramming and Cociu (1991).

ECONOMIC IMPORTANCE

World plum production raised from 8.1 million tons in 1999 over 9.9 million tons in 2003 to 10.3 million tons in 2008 (FAOStat, 2010, www.faostat.fao.org). However, these data are mostly estimations as official and reliable data only exist for few countries. Moreover, the data sometimes refer to volumes sold on the market, sometimes to the estimated production including direct selling and home gardening. In Germany, about 50,000 tons of European plum are sold by the producer markets annually. In total, about 300,000 tons of plums are harvested including fruits produced for direct selling, brandy production and in home gardens. For Germany, the FAO statistical data refer to the amount sold by producer markets, but for other countries the whole production or just estimations are included. Some countries may even use the data communicated to FAO for political reasons. This example shows the low validity of the data available.

According to estimations, China contributed 5.2 Million tons to the world plum production in 2008. In these statistical data, no differentiation between *Prunus domestica* and *Prunus salicina* is made. In China, the vast amount of the production is Japanese plum. For *Prunus domestica*, the countries with the highest production are Germany, the

Fig 2 Diversity of plum fruits within *Prunus domestica*. (**A, B**) plum (**A** 'Avalon', **B** 'Jubileum'); (**C-G**) prune (**C** 'Tipala', **D** 'Colora', **E-F** breeding clones from the Hohenheim breeding program, **G** 'Date prune'), (**H**) Damson ('Blaue Zibarte'); (**I**) plum ('Eibensbacher Aprikosenpflaume'); (**J**) prune ('Harbella'); (**K**) Reineclaude ('Reneklod rannyi'); (**L**) Mirabelle (breeding clone 'Wei 80' from the Weihenstephan plum breeding program).

U.S.A., Romania, Bulgaria and Serbia. Within the U.S.A., California is the top producer, most of the production is used for drying. The by far most important cultivar is 'French Prune'. In Germany, about half of the production is used for bakery, about 30% for fresh consumption and the rest for brandy production. In the other countries mentioned, plums are mostly used for brandy production.

During the last decades, the plum production in Bulgaria, Romania and the region of the former Yugoslavia decreased due to the impact of Sharka. New cultivars resistant to this virus disease are necessary and become available due to breeding activities.

BREEDING OBJECTIVES

Modern plum breeding activities aim at the development of cultivars which are adapted to different climates. They should grow successfully in specific localities and give attractive fruits with good quality for profitable marketing. Winter hardiness for northern and lower chilling requirements for southern production areas are important breeding aims. Productivity and resistance must be regarded as well. Very important are the shipping ability and, especially for late ripening cultivars, their long storage ability.

European plums are used for dessert and for fresh consumption but also for canning, processing, drying, cooking and in baking. Some cultivars, e. g. 'Italian Prune' and also 'German Prune', can be used for all of these purposes. These multi-purpose cultivars are popular in Middle and Eastern Europe. They have small fruits because very big fruits cannot be used for baking or drying. In the future, breeding programs will aim to obtain genotypes with bigger fruits and excellent taste for the fresh market supply as well as cultivars with smaller fruits, which have firm flesh, are

freestone and can be used for processing and for bakery. For the fresh market, semi freestone cultivars are acceptable.

Special breeding programmes are necessary for the different purposes. One should take into consideration that the performance of a plum genotype depends, to a large extent, on the climatic conditions where it is grown. Therefore, a transfer of the results obtaining in one region to others is not always possible and more difficult than in many other fruit species. Ideally, plum breeding is located in the region where the plums are grown.

Because of a high degree of heterozygosity and, in case of *Prunus domestica*, its hexaploid nature, it is very difficult to investigate the inheritance of an individual trait in plum. Quantitatively and qualitatively expressed traits are known. The contribution of gene dose effects to the phenotypically visible characteristics of a trait has to be taken into account. Several studies were made concerning the inheritance of individual traits of interest. Most of these studies are found in the older literature. Inheritance studies are time consuming and require accurate planning, data collection and data interpretation. For many characters, such as disease resistance, a system for the classification of the genotypes under investigation in different classes has to be developed in advance. Appropriate statistical methods have to be applied. Often, the collected data are not normally distributed so that non-parametric statistical tests have to be used. If one of the mentioned points is not considered, the conclusions drawn in a study of inheritance are doubtful.

For inheritance studies, a large progeny per crossing combination is necessary. The more descendants can be evaluated the better are the conclusion which can be drawn. For practical use about 100 seedlings may be enough. If just a tendency in inheritance has to be evaluated 50 seedlings of one crossing combination are usually sufficient. The size of the progenies is quite small compared to that one usual in pome fruit breeding. It is much easier to get a large progeny in pome than in stone fruit breeding. Donors for 24 traits are given by Cociu *et al.* (1997)

Climatic adaptation

Plums growing in different areas and some of widespread cultivars such as 'Prune d'Agen', 'Italian Prune', 'Stanley' and 'German Prune' show a high adaptability to different climatic conditions. Nevertheless, in northern latitudes, the cultivation is restricted by climatic factors. Winter starts early and temperatures fall down to –25°C or even lower. Some cultivars are able to withstand even temperatures below –30°C. Breeding for winter hardiness has been an important aim in some countries (Okie 1995). In Russia, cultivars of *P. domestica* like 'Vengerka Moskovskaya', 'Zuysinskaya' and 'Reine Claude Reform' were used as donors of winter hardiness. Eremin set up a large breeding program using intraspecific crossing in order to develop plums tolerant to winter coldness (Okie 1995).

Fluctuating temperatures during winter often cause damages on trees of some plum cultivars. Trees of cultivars developed in continental climate with high frost resistance may be damaged in more maritime areas because the dormancy is broken by fluctuating temperatures. Damages of the bark on the stem, but also on flower buds have been observed. Via stem damages, pathogenic microorganisms (e.g. *Pseudomonas* species) can enter the plant resulting in the dying off of plum trees as happened frequently in some Central European countries during the last years. Frost damage of flower buds has been observed when a warm period in January was followed by very low temperatures in February. For instance, the cultivars 'Čačanska lepotica' and 'Ruth Gerstetter' are very sensitive. 'Italian Prune' and 'German Prune' are known to be frost tolerant.

To a certain degree, the susceptibility to spring frost also depends on the genotype. However, the most important factor is the developing stage of the flower buds of the respective cultivar at the time where the frost event takes place as well as the quality of the flowers which is mainly influenced by the height of the yield in the preceding year. Early flowering cultivars are generally more exposed to spring frost. A direct comparison between the cultivars is only possible when the same flowering stage is considered. This complex system of influencing factors may be the reason why the degree of frost tolerance of respective cultivars given in literature often strongly differs from experiment to experiment. Hartmann (2002) lists the tolerance and sensitivity to frost of many cultivars. Local and old plum cultivars as well as primitive landraces may be donors

Fig 3 Abiotic factors provoke damages on the fruits. (**A**) internal browning due to temperature fluctuations prior to harvest; (**B**) heat damage on unripe fruit; (**C**) heat damage on ripe fruit; (**D**) caverns in the fruit flesh with gum (pectines) production.

of resistance to frost and drought (Paunovic 1988).

Most cultivars of *P. domestica* have a moderate to high chilling requirement. This is positive for frost resistance but gives problems in areas with low chilling. Even the chilling requirements of some Japanese plum cultivars are not fulfilled in subtropical areas. Inadequate chilling results in delayed and abnormal flowering and reduced yield. No source of low chilling requirement is known in *P. domestica*. Maybe interspecific hybridisation with *P. salicina* cultivars will be successful.

Drought and heat resistance are important traits in areas with low precipitation and hot temperatures during summer. *P. microcarpa* is the most resistant species and can be used for interspecific hybridisation. Temperatures of more than 35 °C can result in heat spots on fruits, visible as sunken areas, sometimes found in Japanese plum cultivars but also in weakly coloured European plum cultivars like 'Jalomița' or 'Ersinger' and others. In blue coloured fruits, cells of the underlying flesh may collapse and darken (**Fig. 3a-c**). There are significant differences between the cultivars. The clone 'Hoh 459' ('Ortenauer' × 'Ruth Gerstetter') obtained in the Hohenheim breeding program in Germany (W. Hartmann) showed good stability in fruit quality and was used in the Weihenstephan breeding program.

Due to the change of climatic conditions during the last decades in Europe, an unusually high degree of twin fruit formation was observed. In some years up to 80% of double fruits were observed depending on the variety. Twin fruits are not marketable. For cherries it has been shown that high temperatures of more than 30°C during the flower bud formation are responsible for the development of twin fruits (Roversi *et al.* 2005). During the fruit development in twin plum fruits often one of the twins is dying which causes problems with subsequent *Monilinia* infections. The occurrence of twins largely depends on the variety. 'Stanley' and also 'Čačanska lepotica' tend to the formation of a lot of twins. This may indicate a correlation with high fruitfulness, but in the very productive 'Čačanska rodna' only some twins were observed. The tendency to form twins is inherited by 'Stanley' and 'Čačanska lepotica'.

Yield potential

The yield of a stone fruit orchard is the result of several factors. The most important one is the sensitivity of the flowers of the variety to cool weather conditions at blooming time. For instance, 'Italian Prune' is very sensitive to bad weather conditions during flowering as well as 'Valjevka' and 'Čačanska najbolja', whereas 'Čačanska lepotica' and 'Katinka' are relatively robust. There are some late flowering cultivars in *P. domestica*. The blooming time is the result of an additive gene effect (Hansche *et al.* 1975).

Today, growers only accept cultivars with high and regular yield. Precocity is only a problem in some older cultivars like 'Italian Prune', 'Bühler Frühzwetsche' and 'German Prune'.

Good donors for precocity and high yield are 'Stanley', 'Čačanska lepotica', 'Čačanska rodna' and 'Verity'. A marker for precocity and high yield is the development of flower buds on one year old long shoots. In some cultivars the fruit set is too high, resulting in small, unattractive and tasteless fruits. A fruit thinning may be helpful. However, seedlings with tendency to overcropping should be discarded during the selection process.

Ripening time

In some countries, there is an extensive supply of plum in many years, resulting in low prices mostly at mid-season. A better price is usually realised at the end and especially at the beginning of the harvesting season. Therefore, an extension of the ripening time is desirable. In the northern hemisphere, fruits of *P. domestica* are harvested from the middle of June till the middle of October.

The highest proportion of early ripening descendants can be obtained by crossing two early ripening cultivars. However, the embryos of early ripening cultivars are often underdeveloped or stones are even empty so that the number of seeds obtainable and their germination rate are both very low. Thus, embryo rescue methods are helpful. Alternatively, the early ripening variety is used as father for pollinating a mid season ripening mother variety. A transgression of the ripening time of the father variety is possible. The cultivar 'Ruth Gerstetter' is ripening earlier than its parent cultivars 'The Czar' and 'Bonne de Bry'. It is the earliest ripening variety in European plum with acceptable fruit quality. The breeding of late ripening cultivars is easier. Quite often, seedlings have a later ripening time than the parental cultivars (Hartmann 1994). Cociu (1977) investigated 4,450 plum hybrids; 44% ripened earlier, 46% were intermediate and only 6% had a later ripening time than the parents. According to Hansche *et al.* (1975), the ripening time is determined by several genes or alleles additive in effect. It has to be taken into account that fruits of young seedlings are generally ripening four to eight days delayed compared to grafted trees of the same genotype.

Fruit characters

1. Fruit size

For fresh consumption, the fruit should outweigh 50 g. Best known is 'President', but its taste is only medium whereas the suitability for storage is good when the fruits are harvested not too late. Large-sized cultivars attracting grower's attention are 'Jubileum' from Sweden and the German cultivars 'Tophit' and 'Haganta'. Large-sized cultivars with good fruit quality were released from East Malling (U.K.) as well ('Avalon' and 'Excalibur'), but their productivity is low in case of suboptimal weather conditions during flowering. Sharka resistant cultivars with blue coloured, large and firm fruits are missing.

For processing, the fruits should not exceed a mass of 40 g. Thus, the desired fruit size in Middle Europe and most countries of Eastern Europe is 30–40 g. The fruits of the majority of the recently released cultivars are of this size.

The fruit size is quantitatively inherited. A crossing of small-fruited cultivars among themselves results in seedlings bearing small fruits. In case of both parents having large fruits the progenies will mostly have smaller ones than their parents. Paunovic *et al.* (1968) found only 2.8% of all hybrids bearing fruits larger fruits than the parents.

2. Fruit shape

The fruit shape is not important in countries where people prefer large fruits for table use. On the contrary, the shape is very important in some other countries, e.g. in Central Europe. The fruits must be oblong to elongated like a prune because round fruits are regarded as plums which are not popular in these regions.

In *P. domestica*, a wide range of different fruit shapes exists. Very often, in an offspring of crossings between parents with elongated fruits all different kind of fruit shapes can be observed.

Oval fruit shape was reported to be dominant over round shape (Okie 1995). Indeed, all seedlings with oblate parents showed oblate fruits. Therefore, this trait can be considered to be recessively inherited.

3. Fruit colour

The fruit colour of plums ranges from black to blue, purple, red to yellow, and some cultivars are even bright coloured. The ground colour is often covered with waxy bloom which makes the fruits very attractive. For fresh market the fruit colour is an important trait. The preferred skin colour varies from country to country. In Middle Europe, blue coloured fruits are preferred. A problem is that some cultivars are coloured completely blue up to three weeks before ripening

so that fruits are harvested unripe if the farmer is careless. Yellow fruits should be handled carefully as otherwise, some days later, brown spots may appear on the skin and the attractiveness of the fruits decreases rapidly.

Dark skin colour results from a high content of anthocyans and is inherited dominantly. As cultivars with dark skin are often heterozygote concerning this trait, descendants originating from a crossing of two dark skinned cultivars can show red, green or yellow coloured fruits as well. Cultivars with yellow skin colour are homozygote concerning this trait.

4. Flesh colour

The colour of the flesh ranges from orange to yellow and greenish yellow to white. Red flesh is found only in Japanese plums and in cultivars of *P. cerasifera*. For fresh consumption the flesh colour is more important than for processing. An orange or golden flesh is preferred.

The flesh colour is a very variable trait. Paunovic *et al*. (1968) found that only 57% of the hybrids corresponded to their parents. In 43% of the seedlings, a different colour appeared. A good donor of orange flesh is the cultivar 'Hanita'.

5. Firmness

The texture of the flesh is the decisive factor for the firmness of the fruit. To a large extent, the firmness depends on the ripening stage of the fruit but also on the variety. The texture reaches from very fine to fibrous. In some cultivars the flesh is melting, in others mealy. A sufficient firmness is important for the transportation value. In some cultivars, the firmness can be reduced very fast after harvesting but this depends on the ripening stage.

Sometimes, there is a relationship between the firmness of the fruit and its juiciness. For fresh consumption a certain juiciness is desired. For use in bakeries juicy and soft fruits cause problems. Paunovic *et al.* (1968) report that firmness is the most variably inheritable character in plums. In their experiments, 75% of the progenies had less firm fruits than the parents. Good donors for high firmness are 'Katinka', 'Tegera' and 'Čačanska lepotica'.

6. Stone adherence

Generally, fruits which have a pit free from the flesh are desired not only for fresh consumption but, to an even larger extent, for using the fruits in bakeries and for processing.

There are different stages in stone adherence ranging from freestone to semifree and clingstone. This shows the quantitative character of the trait which may be determined by gene accumulation. There are some reports (Wellington and Wellington 1927; Paunovic *et al.* 1968; Hartmann 2007) that clingstone is dominant over freestone. Donors for freestone are 'Čačanska lepotica', 'Tegera' and 'Katinka' (Hartmann 2007).

7. Fruit damages due to abiotic factors

Abiotic factors such as heat or temperature fluctuations can provoke damages on the fruit (**Fig 3**). The splitting or shattering of the endocarp (stone) in fruits of *P. domestica* has become a problem of economic importance. Moreover, caverns are developing in the fruit flesh, and these caverns can be filled with gum. The early stages in endocarp lignification may be involved in the development of stone splitting. Factors that enhance the fruit size and rapid changes in the weather conditions worsen this disorder. There are genetically determined differences between the cultivars but fundamental research on this subject is outstanding. In future breeding programmes, a low tendency to endocarp splitting and to gummosis in the fruit flesh should be considered as an important selection criterion.

8. Taste

The most important aspect of fruit quality is the taste. The preferred taste varies from consumer to consumer and from country to country. In South Europe and also in Asia, most people prefer sweet fruits. In other countries cultivars holding a good balance between sugar and acid content are favoured. Fruits with a distinct flavour and firm fruits that soften prior to consumption are desired.

High fruit quality and taste go along with high sugar content which can be estimated by measuring the soluble solids content (SSC). In plums, there is a wide range from 12 to 32% Brix. Prunes are higher in sugar content than plums. Late ripening cultivars usually have a higher SSC than early ripening cultivars. In order to obtain a good fruit quality, a minimum of sugar content is necessary. In late ripening cultivars the SSC should be more than 17% Brix. The perception of sweetness depends on the SSC/acid ratio. The most tasteful cultivars show both a high sugar and a high acid content at picking time. After harvest the acid content declines quickly.

The flavour and the aroma of the fruit are determined by a combination of volatiles. In ripe fruits, there are hundreds of volatiles. In plums, there is a wide range in flavour from very poor to very rich. Taste and flavour mostly correlate with the sugar content.

Some cultivars have a touch of bitterness caused by high contents of polyphenols. Tannins impart a unique flavour preferred by some consumers (e.g. the variety 'German Prune') but outstanding levels produce an undesirably bitter flavour. Bitterness is well inherited by 'Čačanska najbolja'. As the taste of a fruit is determined by a complex of different traits its inheritance is complicated. Often, there are some seedlings with good taste among descendants of two parents with poor fruit quality. Donors of high fruit quality are 'Italian Prune', 'Hanita' and 'Harbella', the latter two especially for flavour and fine acid content. Generally, crossings between rich flavoured plums give a high proportion of seedling with a rich flavour.

Breeding for resistance

1. Strategy

There are a lot of different kinds of damages in plum production caused by abitotic or biotic factors. In case there is any kind of variability in the gene pool of *P. domestica* concerning the reaction of the plants to the attack of a respective pathogen or to an abiotic environmental factor causing damages on the tree, the breeding of resistant or tolerant cultivars is, in principle, possible. If there is no variability species of interest, related species should be investigated. They can be used for interspecific hybridisation. Exemplarily, the breeding of plum cultivars resistant to Sharka disease will be described in detail because Sharka is the most important disease in plums. The strategy for the breeding of cultivars resistant to other pathogens/abiotic environmental factors can be derived from these considerations.

There are several steps in breeding resistant cultivars: First of all, genetically fixed differences in the behaviour of single genotypes of the respective species against the pathogen must be detected. The more genotypes can be tested the higher is the probability of finding resistance and/or tolerance. National gene banks can be used for obtaining a broad spectrum of different genotypes. For this kind of large scale testing, a reliable resistance test has to be developed. Resistant genotypes must be selected in order to use them as a crossing partner. In advance or in parallel to a resistance breeding program, the life cycle of the pathogen and the kind of reaction of the plant against it must be investigated. The durability of the resistance has to be estimated. For this purpose, a preferably large number of isolates of the respective pathogen must be used for inoculation tests. The mechanism of resistance or tolerance to the pathogen has to be

Table 1 Tolerance of some cultivars of European plum against PPV according to the symptoms on leaves and fruits (Hamdorf and Hein 1989; Hartmann 1990; Rühl 1994)

Variety	Leaves	Fruits	Variety	Leaves	Fruits
Anna Späth	–	o	Katinka	–	+
Auerbacher	–	–	Victoria	–	o
Bühler Frühzwetsche	o	+	Mirabelle de Nancy	+	+
Čačanska najbolja	+	+	Jalomiţa	–	–
Čačanska rodna	–	–	Ontariopflaume	+	+
Čačanska lepotica	o	+	Opal	+	+
Čačanska rana	–	+	Ortenauer	–	–
Carpatin	–	+	Oullins Reineclaude	+	+
Centenar	–	+	Pitestean		+
Chrudimer	+	+	Presenta	–	+
Czernowitzer	+	+	President	o	+
Elena	–	+	Ruth Gerstetter	–	+
Ersinger	–	+	Sanctus Hubertus	o	+
Fellenberg	–	–	Stanley	o	+
Felsina	–	–	Tegera	–	–
German Prune	–	–	Topend	+	–
Green Gage	–	o	Tophit	o	o
Harbella	o	–	Topper	o	+
Haganta	o	o	Topfive	–	+
Hanita	–	+	Valjevka	o	+
Haroma	o	+	Valor	+	–*
Herman	o	o	Zimmers Frühzwetsche	–	–

– sensitive (strong symptoms on the leaves/fruits); o weakly sensitive/slightly tolerant; + tolerant (very few symptoms on the leaves/fruits); * During the 1980s, 'Valor' was considered to be fruit tolerant. During the last years, the variety suffers more and more from Sharka and shows symptoms on the fruits.

described. By analysing progenies originating from different crossing combinations between resistant donors and other genotypes, the genetic determination of the resistance trait can be ascertained. If interspecific hybrids have to be used, hybridisation methods have to be developed as far as they are not yet available, e. g. embryo rescue techniques. Before releasing a new variety, the respective genotype has to be tested under natural inoculation conditions on different sites for several years.

2. Sharka resistance

The Sharka disease, for the first time described by Atanasoff (1935), is the most important disease in stone fruit production. It affects European (*P. domestica*) and Japanese (*P. salicina*) plum, peach (*P. persica*) including nectarine, apricots (*P. armeniaca*), sloe (*P. spinosa*), myrobalan (*P. cerasifera*) and, with minor impact, cherry (*P. avium* and *P. cerasus*). It is caused by a Potyvirus, the *Plum pox virus* (PPV). The most eye-catching symptoms are chlorotic rings and/or spots on the leaves of sensitive genotypes. Symptoms on the fruits are depressions on the surface and/or spots or rings which are especially well visible after removing the bloom of the fruits. PPV-infected trees of many cultivars show premature fruit drop. The fruit quality is low because of a high acid and low sugar content. The vegetative growth can be reduced. The lignification process is also influenced which results in a poor elasticity of the shoots. Infected trees are detectable by the easy breaking of the shoots compared to healthy ones. This method can be used as a pre-test for Sharka infection even in leafless trees.

Multiple factors, like biotic and abiotic environmental conditions, the degree of resistance or susceptibility, of tolerance and sensitivity of the respective cultivar, the virus strain or isolate and the age of the trees at the time of its infection with PPV, influence the expression of PPV symptoms. If an old tree gets infected, the infection often remains limited to one or several branches of the tree whereas the infection usually gets fully systemic if a young tree becomes infected. Sensitive genotypes can even die due to PPV infection. Yield losses, poor fruit quality and losses of trees are the most important economic impacts of PPV infections in stone fruit orchards. A detailed description of PPV symptomatology is given by Németh (1986).

There are two ways of avoiding economic damages caused by Sharka: (1) avoiding the infection of the trees with PPV, and (2) using cultivars which show only mild or no symptoms after PPV infection (tolerant and/or resistant cultivars) (terminology used according to Cooper and Jones 1983). The avoidance of infection could be carried out by the use of immune genotypes or by using cultivars and rootstocks which are resistant to the aphid vector of PPV. However, neither immunity nor vector resistance was found within European plum (Rühl 1994; Hartmann and Petruschke 2000; Grüntzig *et al.* 2001; Hartmann and Neumüller 2006). Therefore, breeding programs worldwide focus on gaining tolerant or resistant cultivars. To a great extent, the degree of this tolerance or resistance depends on environmental factors and on the virus isolate infecting the plant. For several years even widespread cultivars which were known not to show remarkable symptoms on fruits or to be quantitatively resistant have suffered more and more from Sharka disease. Therefore, PPV causes increasing economic damage.

The terms "tolerance" and "sensitivity" describe the phenotypically visible reaction of the plant against infection with a pathogen. Tolerant genotypes show no or only mild symptoms. For fruit growers, it is most important that there are no symptoms on the fruits, they therefore often prefer fruit tolerant cultivars which can show symptoms on the leaves but the fruits are only less affected by the pathogen (see **Table 1**). "Resistance" and "susceptibility" are corresponding terms describing the behaviour of the pathogen within the plant. In resistant cultivars, the virus concentration is lower than in susceptible ones and/or the systemic distribution of the virus within the plant is prohibited. For the evaluation of the resistance to PPV, the determination of the viral concentration (e.g. using ELISA or RT-PCR-Techniques) is necessary whereas the tolerance can be estimated just looking at the phenotype of PPV infected plants.

There are two kinds of resistances to PPV known in *P. domestica*: the so-called quantitative resistance and the resistance mediated by hypersensitive response. Quantitatively resistant cultivars have been known since a long time. The virus concentration in the leaves is diminished. However, they can get infected with PPV in the field by aphid transmission. The hypersensitivity resistance, which was discovered later, leads to a complete field resistance of the respective genotype: Trees remain free from PPV in the orchard even under high infection pressure. Therefore,

hypersensitive genotypes cannot be a source of inoculation by PPV in the field.

In order to test the Sharka resistance of *Prunus* species, the following parameters must be taken into account:

- Until now, seven different strains of PPV are known (PPV-D, PPV-M, PPV-Rec, PPV-W, PPV-EA, PPV-C, PPV-T (James and Varga 2004; Myrta *et al.* 2006; Serçe *et al.* 2009). Each strain itself consists of different PPV isolates. Each of these isolates can influence the viral concentration in the plum tissue and the development of symptoms on fruits and leaves of a given plum genotype. Thus, the choice of the PPV isolate for resistance tests can influences its result. Ideally, isolates which usually reach a high viral concentration should be preferred (e. g. the isolate 'CG' described by Kegler (1990). In resistance tests, usually one isolate is used for all the seedlings. The most promising ones have to be tested with a broad range of isolates of each PPV strain in a second step.
- As the inoculation of woody plants using PPV containing plant sap extract is very difficult and the results obtained with this method are not consistent, only the transmission using either natural vectors (aphids) and the transmission by grafting are possible. The aphid transmission in the greenhouse mimics the natural transmission of PPV but it is time and labour consuming and needs a lot of experience in case the results obtained should be reliable. The testing under natural inoculation conditions in the orchards as the only testing method is insufficient: It is well known that some individual trees of even highly susceptible cultivars (e.g. 'German Prune' or 'Auerbacher') can remain free from PPV over a long period whereas all the trees of the same cultivar surrounding got infected. Thus, the testing in orchards under high natural infection pressure cannot be used as test system for resistance screening purposes. Unfortunately, many investigations have been based on this method in the past (e.g. Minev and Dragoiski 1995). The results obtained with this method are more or less worthless, especially if conclusions concerning the choice of parents for resistance breeding are derived from that investigations (e.g. Lahmatova *et al.* 1998). There are different kinds of grafting suitable for inoculation. Often the chip budding method is used: Chips of budwood cut from PPV infected trees are budded into young plants of the genotype of interest. Depending on the number of chips, the PPV concentration within them and the size of the tree to be inoculated, the results obtained with the chip budding method can vary. Therefore, the grafting of budsticks of the genotype of interest onto heavily infected trees in the orchard, the grafting of them onto a virus-free myrobalan rootstock with PPV infected interstem or the grafting on PPV infected rootstocks in the greenhouse are the methods of choice. For testing on hypersensitivity resistance (see below), these methods are necessary for phenotyping the response of a genotype to PPV infection and for determining the degree of hypersensitivity. The bigger the plant and the smaller the inoculum, the lower and slower is the reaction of the plant to the inoculation. For getting fast and clear results, the double grafting method or the grafting on PPV infected trees should be used as there is a continuous virus transport from the infected interstem or rootstock to the scion part.
- The best time for the rating of the symptoms is in late spring time because during summer, the symptoms on the leaves may be masked. Viral concentration within the leaves may decline during high temperature phases so that ELISA tests work best in late spring as well. In order to be able to compare the results of different experiments, some standard cultivars have to be used in each resistance test: the PPV sensitive cultivars 'Italian Prune', 'Čačanska rodna' and 'German Prune', the tolerant cultivar 'Opal', the quantitatively resistant variety 'Čačanska najbolja' and the hypersensitive cultivar 'Jojo'.
- The time of inoculation during the phenological development of the plant has got high impact on the expression of symptoms. Inoculations by chip budding or aphids during summer or in the autumn usually provoke the development of symptoms not before the next spring.
- Using the double grafting method usually one growing season is sufficient for getting reliable results. However, in some genotypes, the Sharka virus remains latent for a few years after inoculation especially when existing trees are inoculated in the orchards (Kegler 1990). Thus, at least those resistance screenings which were aimed to describe the viral impacts on the fruits should be done over a period of at least five years. If the double grafting method is used, usually one growing period is sufficient for getting reliable results.
- The more trees are tested the more meaningful are the results. In practical use, three plants per genotype in greenhouse tests and five plants per genotype in field tests are feasible. Depending on the biotic and abiotic environmental conditions, the reaction of the plant to PPV inoculation can vary. Therefore, the testing on several sites in different geographical regions is recommended.

Most of the reports on Sharka sensitivity or tolerance made in the last decades cannot fulfil all of the mentioned criteria. Moreover, most of them did not determine the resistance of a genotype to PPV but its tolerance or sensitivity because only visible symptoms were rated. Therefore, it is difficult to draw any conclusions concerning the choice of parents for resistance breeding based on these investigations. Only few investigations have been carried out which produced reliable results due to the correct way of testing (e.g. Trifonov 1978; Sutic and Rankovic 1981; Kegler 1990; Petruschke and Schröder 1999). For the selection of parents for breeding Kegler (1990) proposes to use three criteria: a low expression of Sharka symptoms, a low virus concentration within the leaves and a low degree of systemic virus spread within the plant. Genotypes which follow these terms are donors of PPV resistance.

Inheritance of PPV resistance: Most studies carried out during the last decades were dealing with the resistance or tolerance screening of existing cultivars. There are only very few systematic investigations concerning the inheritance of PPV resistance. In many cases, the term "resistance" was used incorrectly instead of "tolerance". Often, only some genotypes of high pomological value were tested instead of whole progenies of several crossing combinations so that no conclusions concerning the inheritance of PPV resistance or tolerance can be drawn. In those studies where whole crossing combinations have been screened, often inadequate test methods were applied. For example Minev and Dragoiski (1995) planted seedlings in a field with heavy infection pressure by aphids and draw conclusions on the inheritance of PPV resistance. However, there was no artificial inoculation of the trees with PPV so that one must doubt the conclusions drawn out of this experiment. Bivol *et al.* (1988) reported the multifactorial inheritance of quantitative Sharka resistance. In most cases, the seedlings were, compared to the parents, intermediate concerning their degree of PPV resistance. Only the combination 'Graf Althans Reneclode' × 'Kirkes' resulted in a higher degree of PPV resistance in some single seedlings. In general, the progenies of the combination of two quantitatively resistant genotypes did not show higher degrees of resistance than the parents.

Until recently, it was assumed that breeding efforts could only result in tolerant and/or resistant cultivars which show only mild PPV symptoms or have a lower virus titer within the plant tissue but always get more or less systemically infected and are, therefore, a source of PPV for the further distribution of the Sharka disease (Atanasoff 1935; Rankovic *et al.* 1995; Lahmatova *et al.* 1998). How-

ever, Kegler *et al.* (2001) and Hartmann (2002) showed that, in *P. domestica*, another type of resistance exists which prevents the systemic infection of plum trees in the orchard by a resistance mechanism mediated by a hypersensitive response (hypersensitivity resistance). The trees of hypersensitive genotypes remain free from PPV in the orchard even if there is a high inoculation pressure by aphids. Thus, they are no source of infection for neighboring plants both in the nursery and in the orchard. In the nursery, only trees free from PPV can develop so that the distribution of PPV over long distances can be avoided. Detailed studies on these resistance mechanisms have been carried out by Neumüller (2005). He described the response of more than 1,150 genotypes of *P. domestica* originating from crossings between sensitive and hypersensitive genotypes at University of Hohenheim to artificial inoculation with PPV using the double grafting method with a PPV infected interstem described by Kegler *et al*. (1994). The inheritance of the hypersensitivity was investigated. In this test system, hypersensitive genotypes show necrosis on the leaves and on the stems as well as the death of young shoot tips. Among the descendants of all the crossing combinations tested, both sensitive and hypersensitive genotypes were found as well as hybrids showing characteristics of both sensitivity and hypersensitivity (Neumüller *et al.* 2005, 2007). There is a smooth transition from sensitivity to hypersensitivity. From the phenotypical point of view, hypersensitivity is a quantitative trait. In order to be able to describe the degree of hypersensitivity of an individual genotype, the ratings of the most important characteristics of hypersensitivity were used to describe the index of hypersensitivity. Hybrids with a similar value of hypersensitivity index were grouped in four classes of hypersensitivity. Only members of two classes are of high pomological value (Neumüller and Hartmann 2008).

Neumüller (2005) investigated the descendants of 26 crossing combinations originating from crossings with at least one hypersensitive parent of *P. domestica*. The hypersensitivity fixed in the Hohenheim gene pool, originating from the crossing 'Ortenauer' × 'Stanley' (e. g. the variety 'Jojo') and effective to all PPV isolates tested up to now, showed a significantly better heredity than the one in descendants of 'K4'-hybrid, the hypersensitivity of which is specific to certain virus isolates. Concerning the percentage of hypersensitive descendants there were major differences in the combining ability of different genotypes. Unexpectedly, crossings between the hypersensitive variety 'Jojo' and cultivars which are of high pomological value due to the excellent taste of their fruits, but highly PPV sensitive (like 'Fellenberg' or 'Felsina') resulted in a high percentage of hypersensitive seedlings. As no maternal effects were observed, it was shown that the hypersensitivity resistance against PPV is encoded in the chromosomal DNA. It can be assumed that the hypersensitivity of European plum against PPV is controlled oligogenically.

The availability of hypersensitive genotypes provides, for the first time, the opportunity of reliably preventing the spread of Sharka virus into areas that have been free from PPV so far. For regions where PPV is prevalent, the cultivation of hypersensitive genotypes is the only possibility of not only minimising the economic damage caused by Sharka disease but also of avoiding it. The results presented concerning the heritability of the hypersensitivity show how to use this mechanism of resistance for breeding new cultivars efficiently. Presently, the breeding of cultivars hypersensitive to PPV is the most promising approach for solving the problem of Sharka disease. In this respect, interspecific hybridisations for producing hypersensitive rootstocks have to be taken into account. Hypersensitivity might also be a promising tool for solving the Sharka problem of species related to the European plum like Japanese plum, peach and apricot.

3. Bacterial cancer

The bacterial cancer (*Pseudomonas syringae* van Hall) is an important disease in most of the plum producing countries. Plums propagated on peach rootstock seem to be less susceptible than those on plum, and myrobalane seem to be less susceptible than 'Marianna' rootstocks (Ramming *et al.* 1991). Nothing is known about the inheritance of the resistance to bacterial cancer in plum. Independently of the genotype used as rootstock, the most important cultural practice for avoiding tree losses caused by *Pseudomonas* and other wound parasites is to avoid damages on the stem (Hinrichs-Berger 2004). It remains important for future breeding work to find sources of resistance against bacterial cancer. In cherry, a resistance test was developed which probably could be adapted to plum (Santi *et al.* 2004).

4. Brown rot

The brown rot, caused by the fungi *Monilinia* spec., is one of the most important diseases of plums. It causes severe losses of the fruits especially in years with a lot of rain. Minoiu (1997) lists some cultivars which are quite resistant to brown rot in Romania (e.g. 'Scoldus', 'Anna Späth', 'Prune d'Agen', 'Blue free', 'Bonne de Bry', 'Ruth Gerstetter') in contrast to some susceptible ones (e.g. 'Ontario', 'Kirke', 'Emma Leppermann', 'Early Laxton'). However, systematical and comparative resistance screenings in plum are missing. Pascal *et al.* (1994) and Walter *et al.* (2004) present two inoculation methods for the screening of apricot, Japanese plum and peach to *Monilinia laxa*. They conclude that the resistance of the fruit flesh does not correlate with the resistance of the fruit skin (epidermis), but both parameters should be considered in resistance tests. One hybrid between *P. salicina* and *P. cerasifera* is described as quite resistant to inoculations into the flesh whereas the investigated cultivars of *P. salicina* are more susceptible. Walter *et al.* (2004) describe methods for resistance tests in apricots which could be used for resistance screenings in plums as well. However, a lot of impact factors such as local humidity, temperature and the strength of inoculum influence the screening results. Differences between the years are often larger than those between different cultivars. In general, cultivars with high sugar content are more susceptible to *Monilinia* infections than others, probably because of their higher tendency to cracking and because of better conditions for the fungal growth. *M. laxa* and *M. fructigena* can only infect the fruit when it is bruised. The fungus itself is not able to overcome the physical barrier (wax layer, cuticle, epidermal layer) which protects the fruit against the environment. Maybe its structure and durability can be used as a selection criterion for *Monilinia* resistance. No data are available on the genetic determination of brown rot resistance.

CLASSICAL BREEDING

Blooming time

Plums are flowering early in the season. Szabó (1989) observed an average interval of eight days between the time of full bloom of the earliest and the latest flowering cultivars in Hungary. However, the blooming time depends also from the region. In warmer regions, the time span between the full bloom of early and late blooming genotypes is longer than in cooler or in continental climate. Nicotra *et al.* (1983) report that, in Italy, the variety 'Valor' started blooming 22 days before 'Jefferson'. The blooming times of important European plum cultivars are given in **Table 2**.

The blooming time of the individual flower depends on the position of the flower bud on the tree. Unlike most of the older cultivars of European plum, new cultivars usually set flower buds on long shoots. These flower buds are two or three days delayed in blooming time compared to the flowers developing on short shoots growing on two or three

Table 2 Blooming time of some European plum cultivars.

Very Early	Early	Medium	Late	Very Late
Czernowitzer	Avalon	Bühler	Anna Späth	Blue Bell
Lützelsachser	Čačanska. najbolja	Čačanska lepotica	Auerbacher	Italian Prune
Wilhelmine Späth	Čačanska rana	Čačanska rodna	Čačanska late	Pitestean
Zwintschers Frühe	Dabrowice	Ersinger	Carpatin	
	Haroma	Excalibur	Centenar	
	Jojo	Hanita	Elena	
	Jubileum	Hanka	Gabrowska	
	Opal	Katinka	German Prune	
	Ortenauer	Top	Harbella	
	Presenta	Top 2000	Herman	
	President	Topfive	Mirabelle	
	Ruth Gerstetter	Topking	Stanctus Hubertus	
	Tegera	Topper	Stanley	
	Tipala		Tophit	
	Valor		Tuleu Gras	
			Valjevka	

Table 3 Fertility of European plum cultivars.

Self-fertile		Partial self-fertile	Self-sterile
Auerbacher	Katinka	Bluefre	Avalon
Bühler Frühzwetsche	Nancy Mirabelle	Čačanska rana	Excalibur
Čačanska lepotica	Presenta	Čačanska najbolja	Green Gage
Čačanska rodna	Stanley	Chrudimer	Lützelsachser
Elena	Tegera	Ersinger	Magna Glauca
German Prune	Top 2000	Italian Prune	Opal
Hanita	Topfit	Jubileum	Ruth Gerstetter
Harbella	Topfive	Ortenauer	President
Haroma	Topking	Tophit	Valor
Herman	Topper	Voyageur	Zimmers Früh-zwetsche
Jojo	Valjevka		

years old shoots. The delay in blooming time on long shoots ensures a better fruit set because at least some flowers can escape from bad weather conditions on individual days during the blooming period and undergo the usual pollination and fertilisation process. Fruit originating from flowers on long shoots are ripening a bit later than the other ones.

The length of the flowering period is genetically determined but largely modified by the environment as well. Szabó (1989) subdivides the cultivars into three groups depending on the length of their flowering time: short (less than 8 days), intermediate (8–11 days) and long (more than 11 days).

Fertility

The fertility is genetically determined. Most of the cultivars of *P. salicina* and all American species as well as their intraspecific hybrids are considered for practical use as self-sterile, but there are some new fertile or partial self-fertile Japanese plum cultivars introduced in the last years (Ramming 2006). *P. cerasifera*, also a diploid species, cannot be considered as entirely self incompatible, but fruit set after self pollination is low (Shoferistov 1986). In European plum, self-fertile, partial self-fertile and self-sterile genotypes are known (**Table 3**). The extent of self fertility is a result of different external and internal factors and depends, to a high degree, on the flower quality as well as on the temperature. The temperature influences the speed of the pollen tube growth but also the aging of the ovule.

Fertility tests are made by isolation of branches and self crossing. Tests for partial self-fertility are made by cross pollination in comparison to self pollination. For the assessment of the fertility of a respective genotype investigations over a period of more than one year are necessary. Pollination and fertilization are necessary for fruit set in plums. Parthenocarpy has never been observed under natural conditions. The fertility of any plum is expressed by the fruit set. According to their fruit set after open pollination, Szabó (1989) assigned 58 European plum cultivars to four groups (**Table 4**). The fruit set which is best in orchard conditions depends on the degree of the flower set and on the fruit size of the respective cultivar. It varies between 10% for genotypes with large and 20% for those with smaller fruits. After cross pollination in the breeding process the fruit set can be higher and may reach more than 50%.

Table 4 Groups of self-compatibility and fruit set in plum cultivars (according to Szabó 1989).

European plum			Japanese plum	
Group	**Fruit set**	**Frequency of cultivars**	**Group**	**Fruit set**
Low	< 10%	10.3%	Low	< 5%
Intermediate	10–20%	22.4%	Intermediate	5–10%
High	20.1–40%	54.0%	High	> 10%
Very high	> 40%	10.3%		

Intersterility

Cross incompatibility prevents a fertilisation between special combinations of plum cultivars. Among European plums, only very few reports on intersterility are known. Tehrani (1972) found incompatibility between some closely related cultivars bred at Vineland Station. The pollen tube growth is influenced by S-alleles. Other than in *P. avium*, only few data are available for S-loci in *P. domestica*. The S-locus consists of two genes, the *S-RNase* gene and the *SLF/SFB* gene. The S-RNase is the female determinant and is secreted in large amounts into the extracelluar matrix of the style. SLF/SFB, the male determinant, is a member of the F-box family proteins; it is responsible for the degradation of RNA in incompatible pollen tubes (Takayama and Isogai 2005). Sutherland *et al.* (2004, 2008, 2009) developed a system for investigating the S-loci of *P. domestica*. For S-RNases of two European plum cultivars ('Verity' and 'Blue Rock') and three myrobalane genotypes, they found a 97% identity with S-RNases from other *Prunus* species such as *P. avium*, *P. salicina*, *P. dulcis* and *P. mume* on the protein level and developed primers for amplifying poly-

morphic *SFB* alleles, a technique which could be used for studying the taxonomy of *Prunus* species and the function of selfcompatibility and selfincompatibility in *P. domestica*. However, the hexaploidy complicates the understanding of S-allele interaction in the style as shown for the tetraploid *P. cerasus* (Hauck *et al.* 2002). In several studies, intersterility with 'Italian Prune' was observed (Lee 1981; Tehrani 1991). In contrary, intersterility has never been observed in the extensive breeding program of Hohenheim University including several fertilization studies (Hartmann and Stösser 1994). Probably, the observation can be explained by the sensitivity of the variety 'Italian Prune' variety to low temperature which influences the fertilization success. Szabó and Nyeki (2000) reported about the cross fertility of some European and Japanese plum cultivars. Due to its hexaploidy, intersterility plays a minor role in European plum. In Japanese plum, intersterility occurs more often.

Sterility

A low fruit set may be the result of morphological sterility based on short style, small stigmata or underdeveloped ovary. Over a period of more than 20 years, Surányi (1994) explored the flower anomalies of plums and found that the traits of sterility are inherent but that there are also seasonal effects. On young plum trees, more sterile pistils are found than on older trees. The low fertility of seedlings in the first or second year of flowering is based on the ontogenesis of the plant. It is typical for the transition period between the juvenile and adult period. Male sterility is known in plums since Crane (1925) reported about this phenomenon in the variety 'Gold Esperen'. The Romanian variety 'Tuleu Gras' is male sterile as well. This male sterility is inherited dominantly. Fifteen cultivars introduced in Romania are male sterile, some of them are the most valuable, e. g. the variety 'Pitestean' (Botu *et al.* 2001).

Pollination

Pollination is the transfer of the pollen to the stigma. In cross breeding this is only possible with cultivars of nearly the same blossom time. Stösser (1985) found a decline in the fruit set when pollination was made after the fifth day of flower opening. In practical breeding, the best time for pollination is in the first two days of the opening of the flower. If there is a requirement of crossing between cultivars with larger differences in blooming time there are several possibilities: One can use trees growing in different regions with different climatic conditions, branches of the male variety can be cut and put in a warm chamber (20°C) to enhance flower development, or the pollen of the earlier blooming variety is stored in a refrigerator.

There is no loss of viability during the storage of pollen at 4°C for one week. Lorenz (2000) found a decline of 30% in pollen germination after a storage time of 2–3 weeks at 4°C. Using pollen stored in evacuated glass tubes at –1 to –20°C for one year, Lee *et al.* (1981) observed good pollen tube growth. This may be an interesting method for pollen conservation.

The results of cross pollination depend much more from the female parent than from the quality of the pollinator. Good pollinators within the European plum are, e.g., the cultivars 'Stanley' and 'Čačanska lepotica'. Good pollinators produces about 50,000 pollen grains per flower. In 'Stanley' and 'Italian Prune', more than 70,000 were found (Hartmann *et al.* 1994).

In crossing experiments, the quality of the pollinator must be considered. The quality of the pollen depends on the deposition of starch. The highest content was found just before the opening of the flower; high starch content in the pollen grain correlates with the speed of the pollen tube growth (Lorenz 2000). Therefore, for collecting pollen for use in crossing, flowers of the male parent should be picked just before opening (in the so-called balloon stage). The best time for the pollination is one to three days after the opening of the flower. In this case, flowers of self-fertile genotypes must be emasculated before the pollen is ejected. Under field conditions, emasculation results generally in a poorer fruit set (Kellerhals and Rusterholz 1994).

Therefore, a procedure was developed which avoids the need for emasculation in many cases: Petals of flowers in the balloon stage are removed one or two days before the opening of the flower. At this phase of flower development, a self pollination is not possible but the stigmata are already receptive for foreign pollen. The pollen is transferred to the stigma using a fine brush. After the pollination, the branches with the pollinated flowers must be isolated in order to avoid the uncontrolled pollination by insects. Bags of synthetic, white material with a diameter of 20–30 cm and a length of 40–50 cm can be recommended. The duration of flower isolation depends on the weather conditions and should be at least one or up to two weeks.

Germination

A stratification of the seeds at 4–5°C for 3–4 months is necessary because their dormancy has to be overcome. Stratifying more than 10,000 seeds directly after harvesting, Jakubowsky (1998) achieved an average germination rate of 33% on the average of six years. Results varied remarkably from year to year: Whereas 59% germinated in 1996, only 20% germinated in 1991. A main problem in the germination process is the thickness of the stones. The germination of some seeds may be delayed for one or two years. Paunovic *et al.* (1968) obtained 976 seedlings out of 4,284 seeds (22.8%), and only 11.3% reached the adult phase. As the labor costs for pollination are very high in *Prunus* species, these low germination rates are not satisfying.

Theiler (1971) developed a special method of embryo culture for cherries. This method was successfully used for plums and prunes (Hartmann 1994). Stones are carefully cracked using a bench vice. For swelling, the seeds are incubated in tap water or a fungicide solution over night. The testa and residues of the endosperm adhering at the embryo must be removed using pincers or finger nails. The embryos are sown in a sterile substratum containing peat, sand and perlite. For optimum growth of the seedlings, temperatures of 25°C for 16 hours during the day and 15°C during the night are recommended. The germination step should be done in a climatic chamber, but a heated greenhouse with additional light can be used as well. The application of fungicides may be necessary to prevent fungal infections of the young embryo. Within a week, the cotyledons get green and the radicle starts growing. About three weeks after sowing, the young plants are transplanted into bigger pots and transferred to a greenhouse. Using this method, the germination rate is very high (up to 90%). The germination of the embryos can be started immediately after harvesting the fruits without the need for stratification. Under good cultivation conditions using additional light for enhancing the plant growth development, the young seedlings can reach a height of 150 cm till the end of the year of harvesting the fruits.

Prior to embryo culture, the stones can be stored for several months under dry conditions at about 10°C. There are also some other germination techniques, for example the 'hot chilling' method: Therani (1991) obtained quite high germination rates when keeping the seeds for a time of 3 weeks at 21°C and, afterwards, at 5°C. Germination started three months later. In the Weihenstephan breeding program, high germination rates were obtained with the following method for sawing *in vitro*: The stones are cracked and the seeds are soaked in tap water until they are swollen. Afterwards, they are surface sterilised (20 min in 1.5% NaOCl plus small amounts of Tween® 20) and put into MS medium (Murashige and Skoog 1962) supplemented with 1.44 µM 6-benzylaminopurine. They are stored for about 12-16 weeks at 4°C in a cool chamber in the darkness. After that, they are transferred to the tissue lab (photoperiod of 16 h) at 20°C. A few weeks later, the embryos start growing. The

Table 5 Culture media for *in vitro* propagation and rooting of *Prunus domestica* genotypes. (BMV/2: Koubouris and Vasilakakis (2006), modified; C_2d: Chée and Pool (1987); WPM: Dimassi-Theriou (1995), modified). Plants growing on C_2d culture medium have less chlorophyll and produce smaller and more compact tuffs than on BMV/2 medium, but multiplication rate is the same or even higher.

Medium	BMV/2	C_2d	WPM
Used for:	**Proliferation**	**Proliferation**	**Rooting**
Macro-salts (mg/l)			
KNO_3	800	1 900	
NH_4NO_3	400	1 650	400
$Ca(NO_3)_2 \cdot 4H_2O$	300	708	556
$CaCl_2 \cdot 2H_2O$	220		96
$MgSO_4 \cdot 7H_2O$	370	370	370
KH_2PO_4	500	170	170
K_2SO_4	400		990
Micro-salts (mg/l)			
$MnSO_4 \cdot 4H_2O$	11	1.115	22.3
$ZnSO_4 \cdot 7H_2O$	4	8.6	8.6
H_3BO_3	3	6.2	6.2
KI	0.3		
$CuSO_4 \cdot 5H_2O$	0.03	0.025	0.25
$Na_2MoO_4 \cdot 2H_2O$	0.3	0.25	0.25
$CoCl_2 \cdot 6H_2O$	0.03	0.025	
$FeSO_4 \cdot 7H_2O$		27.85	27.8
$Na_2EDTA \cdot 2H_2O$		37.25	37.3
NaFeEDTA	30	29.360	
Vitamins and other organics (mg/l)			
Inositol		1001	
Thiamin·HCl	1	2.5	1
Nicotinsäure	1	0.985	0.5
Pyridoxin·HCl		0.846	0.5
glycine	2		2
Myo-inositol	0.5		0.1
Ca-DL-pantothenate	0.5		
biotine	0.1		
p-Aminobenzoic acid	1		
folic acid	0.01		
riboflavine	0.1		
Sugars (g/l)			
saccharose	13	30	30
sorbitol	11		
Phytohormones (mg/l)			
Indole-3-butyric-acid			1
6-Benzylaminopurine	2	1.128	
Gibberellic acid (GA_3)	3.949		
Indole-3-acetic-acid	0.1		
Agarose (g/l)	6	9	6
pH (prior to autoclave)	5.2	5.7	5.8

young plants are adapted to soil conditions in the greenhouse after they have developed several leaves. As it is very time consuming, this method should only be used if direct sawing cultivation in the greenhouse is less promising due to suboptimal conditions (e. g. heat, low air moisture).

The seeds of early ripening cultivars are often imperfectly developed. Therefore, the germination rate is usually very low. *In vitro* embryo culture was developed for *P. salicina* and successfully applied by Bellini and Nencetti (1998). Gerecheva and Zhivondov (2002) describe an embryo rescue method. In expermiments with the cultivar 'Burmosa' (*P. salicina*), the germination rate was 70–100%. The adaptation of the medium composition and the culture conditions is necessary. The smaller the embryos the higher the demand on the composition of the culture media (Ramming 1990). Embryo rescue techniques are also applied to seeds obtained from intraspecific crossings in case the seeds are not fully developed. In the Weihenstephan breeding program, the use of C_2d-culture medium (Chée and Pool 1987) supplemented with 1128 µg/l 6-Benzylaminopurine gave the best results in *in vitro* culture of immature embryos (**Table 5**).

Cultivation of seedlings

Seedlings obtained using the embryo culture method avoiding stratification as described above may stop growing after 4 to 6 weeks. After spraying gibberellic acid (GA_3, 0.5 g/l, in 50% (v/v) ethanol) the terminal bud starts growing again. Additional light during the cultivation is very useful for a good development. Alternatively, the seedlings can also be exposed to light for 24 hours per day, then the application of gibberellic acid is usually not necessary.

When the seedlings have reached a height of more than 50 cm they can be planted directly in the field, otherwise one year of cultivation in the nursery is recommendable. Furthermore, good horticultural practice (fertilization, irrigation, pesticide/herbicide treatments etc.) should be applied during the following years in order to enhance the vegetative growth of the seedlings. In this way the juvenile period can be overcome as soon as possible. Attention should be paid for aphid and especially mite control. In some years, the vegetative growth is strongly reduced by the mite species *Aculus fockeui*.

The seedlings are planted in the field at a distance of about 4×1.25 m. The better the seedlings grow the earlier the first flowers appear. Depending on the crossing combination individual seedling may flower as early as in the second year. The majority of seedlings will remain in the juvenile phase for about four years. Very often, the zone which remains juvenile and never sets flower buds is very small compared to other fruit trees such as apple or pear (**Fig. 4**). Even on thorns which appear during the second and third year in the life cycle of a seedling often set flower buds. Therefore, the grafting of seedling budsticks on dwar-

Fig. 4 Seedling 'Wei 170' ('Hanka' × 'Čačanska rana'). The seed was sown in September 2005, the picture was taken in April 2009. The juvenile zone where no flowers developed is very small, even side branches with low insertion are flowering.

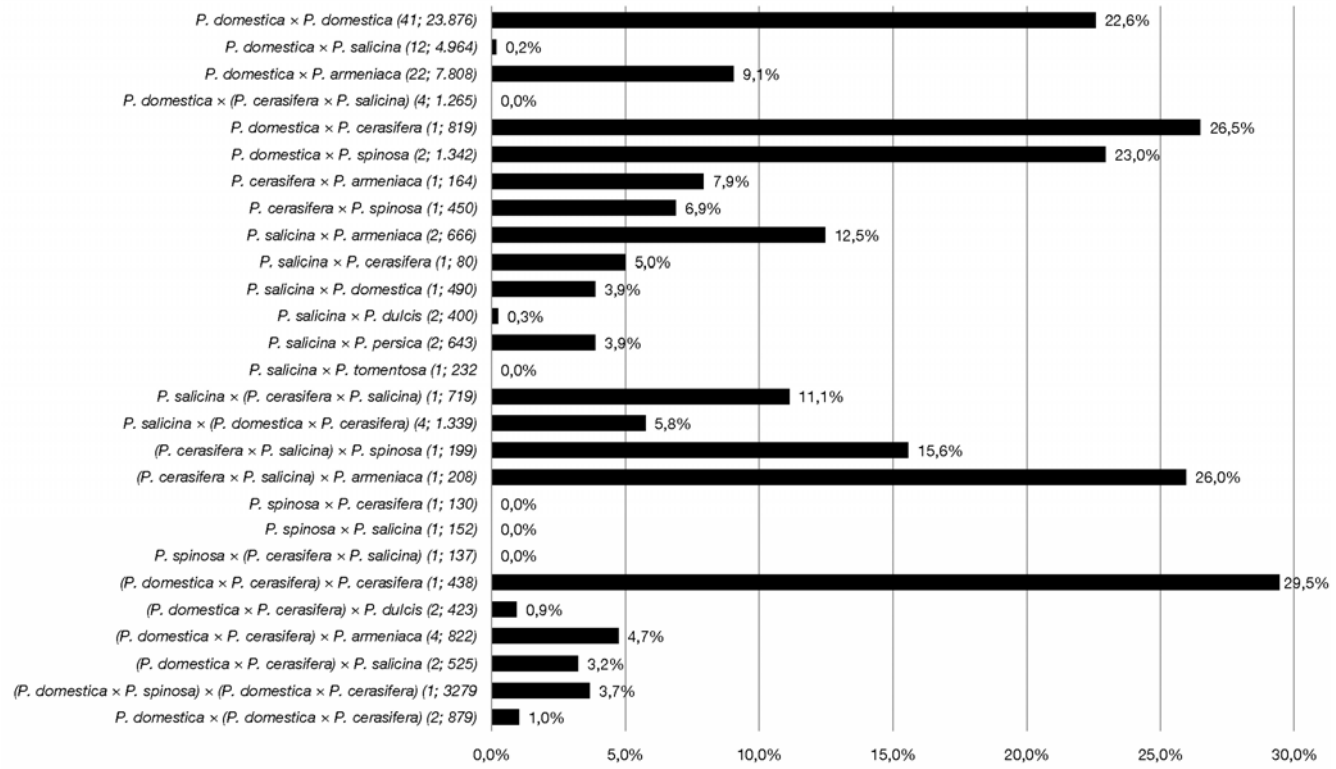

Fig. 5 Fruit set (in % of pollinated flowers which develop into fruits) in several interspecific crossings made in 2006 and 2009 at Weihenstephan. The numbers in brackets give the number of crossing combinations and the number of totally pollinated flowers, respectively.

fing rootstocks as recommended in apples or pears for earlier flowering is not necessary.

Sporadically, genetic dwarfism, chlorophyll deficiency and albinism or fasciated growth forms can be observed. Such genetic defects occur much more frequently after self pollination (inbreeding depression). Mostly, those seedlings are of low vitality and only bear rarely fruits in case they survive during the first years at all. Therefore, they can be discarded before planting into the field.

Interspecific hybridization

P. domestica itself is considered to be a hybrid between *P. cerasifera* and *P. spinosa*. However, this hypothesis is often challenged. Moreover, the botanical systematic of the genus *Prunus* is complicated and unclear. Nevertheless, so called interspecific hybrids are of importance in plum breeding. For the improvement of rootstocks, methods of interspecific hybridisation between different species of the genus *Prunus* are commonly used. For example the rootstock 'Marianna' is an interspecific hybrid between *P. cerasifera* and *P. munsoniana*. For scion breeding, the impact of interspecific hybrids is, up to now, comparatively low. Only some hybrids between *P. salicina* and *P. armeniaca*, known as plumcots are of commercial interest. Any interspecific hybrids between any species of plum and the apricot are called plumcots. Most of the existing plumcots are hybrids of *P. salicina* or *P. cerasifera* with apricots (*P. armeniaca* or *P. mume*) (Okie 1995). Okie (1995) gives a short overview of the history of plumcots.

Interspecific hybridizations enable the possibility of transferring important traits which only occur in one species to another one. For example, the cold hardiness of *P. spinosa*, *P. cerasifera*, *P. americana* and *P. ussuriensis* might be transferred to *P. salicina* or *P. domestica*. The high fruit quality of *P. domestica* which is manifested in its high contents of organic acids, sugars and aromatic compounds makes it a promising crossing partner for improving the poorer fruit quality of other *Prunus* species. Moreover, the European plum is the only *Prunus* species with genotypes completely resistant to the *Plum pox virus* mediated by a hypersensitive response. Therefore, it is an interesting crossing partner for introducing hypersensitivity against PPV into other *Prunus* species. Recently, a breeding program with this aim was started at Weihenstephan (Neumüller *et al*. 2009). Genotypes of *P. salicina* excel other species in its fruit size and good transport and storage ability of the fruits. Thus, hybrids between European and Japanese plum seem to be promising in improving the pomological value of both species. Oldén (1965) reports on such hybrids. His findings indicate that it is better to use the European plum as female parent because the fruit set and the embryo quality is much lower in the reciprocal combinations. Self-fertile genotypes of the European plum tend to give higher fruit set than self-incompatible genotypes when hybridized with *P. salicina*. However, there is a specific combining ability for the different genotypes of European and Japanese plum. The fruit set varied in between 0.0 and 19.4 %. In the Weihenstephan breeding program, hybrids of *P. domestica* with *P. armeniaca* gave much better fruit set than crossings with *P. salicina* (**Figs. 5, 6**). This may indicate a quite close taxonomic relationship between European plum and apricot. *P. cerasifera* seems to be a "genetic bridge" between different *Prunus* species. This species is well compatible with most of the other tested *Prunus* species.

The number of chromosomes varies within the genus *Prunus*. The European plum (*Prunus domestica* including *P. domestica* ssp. *insititia*) is hexaploid (2n = 2× = 48), the sloe tetraploid (2n = 4× = 32) whereas the Japanese plum as well as most of the other *Prunus* species belonging to the group of plums are diploid (2n = 2× = 16). Therefore, the chromosome status has to be considered in interspecific hybrids. Detailed investigations concerning this problem have been carried out by Oldén (1965). He found that seedling originating from crosses between hexaploid and diploid species usually showed 32 chromosomes (tetraploid), but sometimes hexaploid, pentaploid and octoploid seedlings occurred. These results were confirmed by Neumüller *et al.* (2009). In general, the vegetative characters of hybrids between *P. domestica* and diploid *Prunus* species were similar to *P. domestica* whereas "the flowers, their arrangement and the fruit characters were intermediate or preponderant

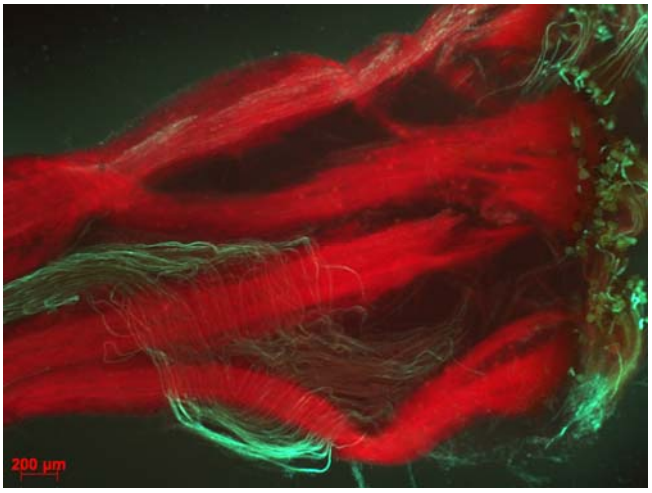

Fig. 6 Stigma and upper part of the style of *P. domestica* 'Hoh 4517'. Pollen grains of *P. armeniaca* 'Mino' have germinated on the stigma and grow through the style. 48 hours after pollination. Stained with aniline blue. Callose of the pollen tube shows greenish-white fluorescence. Chlorophyll fluorescence can be seen in red.

to the diploid parent" (Oldén 1965). The fertility of the hybrids was good. This example shows that interspecific hybridization can successfully be used in breeding programs.

In most cases, hybrids show leave and fruit characters intermediate to those of the parents. Although its chromosomes being very small, the hybrid nature of seedlings with *P. domestica* as one parent can be shown by counting the chromosome number in the roottips of the seedlings and determining in that way the ploidy level. Alternatively, flow cytometry can be applied.

When starting with interspecific hybridization, the germination of the pollen on the stigma of the crossing partner and the growth of the pollen tube should be investigated using a fluorescence microscope (**Fig. 6**). If the pollen tube reaches the ovary and the fertilization takes place, but the fruit set is very low or the fruits have no viable seeds, postzygotic incompatibility barriers occur. They can only be overcome by embryo rescue techniques.

MOLECULAR MARKERS AND GENOME SEQUENCING

In *P. domestica*, no molecular markers for resistance traits which could be used for selection are available. This is due to the hexaploidy of the species which hampers their development. Neumüller (2005) tried to develop AFLP-Markers for the hypersensitivity resistance trait against Plum pox virus and failed due to the probably oligogenic determination of the trait and the hexaploid genome. He resumes that in *P. domestica* it might be more efficient to put effort in the development of test systems or markers which can be applied and rated phenotypically. For diploid myrobabalane, markers for nematode resistance were developed in the French *Prunus* rootstock breeding program (Dirlewanger *et al.* 2004) and applied to interspecific hybrids of myrobalane with almond, apricot and peach (Esmenjaud *et al.* 2009). For *P. domestica*, RAPD- (Gregor *et al.* 1994), RFLP- (Casas *et al.* 1999) as well as SSR-markers (Decroocq *et al.* 2004) are available for molecular genotyping. There have been no efforts to sequence parts of the *P. domestica* genome. Genomic linkage maps have not yet been developed.

MUTAGENESIS, *IN VITRO* CULTURE, AND GENETIC ENGINEERING

Most of the efforts in inducing mutations in stone fruit crops was made on peach, only very few on plum (Srinivasan *et al.* 2005). Johannson and Oldén (1962) describe suitable methods for inducing polyploids, especially for the generation of unreduced gametes during meiosis using colchicine and other mutagenic substances or irradiation. As mutations are often unstable in somatic tissue and tend to result in chimeric plants they prefer to induce mutagenesis during the development of gametes and use them for breeding purposes. In some cases, e. g. for the generation of fertile pollen of triploid genotypes, they obtained good results with the colchicine treatment. There are some reports on the induction of mutagenesis in European plum using x-rays in order to obtain spur types of some plum cultivars (Cociu *et al.* 1997). Nowadays, the induction of point mutations, nucleotide insertions or deletions plays no role in plum breeding because it is not expected that important breeding aims can be achieved by single small-scale mutations of existing cultivars.

There are many reports on the *in vitro* culture of *P. domestica* (Feucht 1982; Jones and Hopgood 1979; Negueroles and Jones 1979; Pietropaolo and Reisch 1984). In our laboratory, the culture media given in **Table 5** give best results for a broad range of *P. domestica* genotypes and interspecific hybrids (multiplication via axillary shoots and rooting, respectively). No reports are known concerning somatic embryogenesis or somatic hybridization within the plum species.

Despite of large efforts, genetic transformation and regeneration in plum has only been successful in few cases: A part of the coat protein gene of the *Plum pox virus* was transferred to the genome of seedlings of the *Prunus domestica* genotype 'B 69 158' (Scorza *et al.* 1994). One of these genetically modified seedlings, the clone 'C5', shows a level of resistance to PPV similar to that of the well-known quantitative resistant cultivars of European plum (e.g. 'Čačanska najbolja'). It is assumed that the resistance of 'C5' which was released in the USA under the cultivar denomination 'Honeysweet' is based on posttransscriptional gene silencing (Ravelonandro *et al.* 1998; Hily *et al.* 2004). However, this kind of resistance is not in advantage of the known quantitative resistance in existing cultivars as the genetically modified plants can get infected with the virus, e. g. when the PPV sensitive rootstock is infected, and can serve as host of PPV. Recent approaches use artificially designed hairpin constructs to induce PPV resistance in *P. domestica*, which was shown to induce post-transcriptional gene silencing directed against PPV in *Nicothiana benthamiana* (Tian *et al*. 2008). The bottle neck in the production of genetically modified woody plants is the regeneration of whole plants out of transformed undifferentiated tissue (Petri and Burgos 2005). In most cases, an efficient rate of transformation and regeneration in *Prunus* species was only achieved when embryonic tissue (i.e. embryos obtained from seeds) were used as base material (Mante *et al.* 1991; López-Moya *et al*. 2000; Srinivasan *et al.* 2005; Petri *et al.* 2008; Tian *et al.* 2008, 2009). The only report on a stable transformation system for a European plum cultivar comes from Russia: Mikhailov and Dolgov (2009) used leaf explants of the Russian cultivar 'Startovaya' to develop a hygromycin selection system.

López-Moya *et al.* (2000) are of the opinion that, for the moment, biotechnological methods cannot contribute to the improvement of plum cultivars concerning the PPV resistance. Currently, other characteristics of plum cultivars are not tried to be genetically modified. Petri and Burgos (2005) consider genetic modification to have a certain value in the amelioration of fruit trees; however they think that this method will not be applicable during the next time. The prerequisite for its successful use would be the development of an efficient transformation and regeneration protocol for a broad range of genotypes of European plum.

Moreover, there is too less knowledge of the genetic determination of agronomic important traits of plums which is necessary for the successful application of gene transfer in practical breeding. Probably, gene transfer will be mostly restricted to plants used for scientific purposes. In this application it can serve well to let understand underlying reasons for physiological processes. At the current state of

knowledge, genetically modified plum cultivars are not necessary for the plum production. Classical breeding methods are far from being the limit of the improvement of plum genotypes.

FUTURE WORK, PERSPECTIVES

There are five main challenges in European plum breeding: (1) The hexaploid genome hampers the study of the inheritance of single traits. If the inheritance of a special character has to be investigated, it will be necessary to reduce the ploidy level by interspecific hybridization with related diploid species and to transfer the trait of interest into a diploid hybrid plant. With the availability of diploid plants the development of molecular markers might become available. Of course, this strategy is not applicable for fruit traits but could be useful for resistance traits. (2) Breeding for resistance will still be predominant. For Sharka containment, hypersensitivity resistance is available. It can be transferred by classical breeding methods. New natural sources of resistance should be found. A pyramiding of different Sharka resistance mechanisms is desirable. For that purpose, genomic markers are necessary. As long as no resistance genes are sequenced, only pathogen derived resistance can be used for developing resistant plants by genetic engineering. Breeding for *Monilinia* resistance is still hampered by the lack of a reliable screening system. (3) Endocarp splitting as well as caverns in the fruit flesh cause more and more economic losses. Detailed studies on the underlying reasons are outstanding. Sources for resistance must be identified. (4) Crossings within the *P. domestica* group may result in an even larger diversity of plum fruits, e. g. crossings between mirabelles and large sized prunes or reineclaudes. Fruit size and firmness can still be enhanced. More and more fruits will be used for fresh consumption. Thus, the market will need a broad range of cultivars with high quality plum fruits (large sized, high sugar content, different skin colours). (5) Via interspecific hybridization, economically important traits present in European plum could be transferred to related species such as apricot, peach and Japanese plum (e.g. hypersensitivity resistance to PPV, resistance or tolerance to European Stone Fruit Yellows (ESFY)). In some cases, interspecific hybrids could be used as rootstocks for related species.

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