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Institute of Agronomy

Title:

# Histochemical location of key enzyme activities involved in stigmatic receptivity of *Matricaria chamomilla* L.

**- Master Thesis -**

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Date:

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## Declaration of autonomous work

I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

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# Abstract

Abstract

It is assumed that German chamomile (*Matricaria chamomilla* L., Asteraceae) exhibits sporophytic self-incompatibility. However, it has not yet been completely clarified if the incompatibility mechanism is indeed located on the stigma surface. In some plants, the incompatibility reactions are associated with inadequate penetration of the pollen through the cuticle of the stigma. For this reason, the activity of certain enzymes, such as cutinases, is believed to play an important role in the incompatibility reactions. The present work has been taken up to understand the relationship between stigma maturity and pollen receptivity in German chamomile. The primary objective was to determine whether the compatibility mechanism has an effect on the activity of the enzymes involved in stigmatic receptivity. Therefore, after repeated manual self-pollination, the interactions between pollen and stigma were made visible by staining enzymatic activities and then recorded under the microscope. Accordingly, stigmatic activity of esterases and peroxidases were identified in different floral development stages and compared between self-compatible and self-incompatible genotypes. In the course of floral development, there was generally a constant increase in stigmatic peroxidase activity, whereas a rather irregular development was observed with regard to the stigmatic esterase activity. Overall, the results show that there were no significant differences between the self-incompatible and the self-compatible genotypes. However, significant differences were found between individual genotypes, but these were rather due to a high genotypic variability and therefore did not occur exclusively between self-compatible and self-incompatible genotypes. Further research is needed to reveal more details about the incompatibility mechanism in German chamomile.

*Keywords:* self-incompatibility, stigmatic receptivity, *Chamomilla recutita* (L.) Rauschert, *Matricaria chamomilla* (L.), *Matricaria recutita* (L.), chamomile

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# 1. Introduction

Pollen-stigma interactions in Asteraceae are associated with a presumably semi-dry stigma surface. Upon contact of the three-celled pollen with the papillae, pollen coat is released. The growth of incompatible pollen is arrested soon after germination resulting in deposition of callose on the pollen tube and papillae (Faehnrich et al., 2015). Not much information is available on the pollen-stigma interactions in German chamomile (*Matricaria chamomilla* L., Asteraceae). It is assumed that chamomile plants exhibit sporophytic self-incompatibility, with the self-incompatibility reaction occurring on the surface of the stigma. However, it has not yet been completely clarified if the mechanism of self-incompatibility in chamomile is indeed located on the stigma surface. In some plants, the incompatibility reactions are associated with inadequate penetration of the pollen through the cuticle of the stigma (Sharma & Bhatla, 2013). For this reason, the activity of certain enzymes, such as cutinases, is believed to play an important role in the incompatibility reactions (Novak et al., 2019).

The present work has been taken up to understand the relationship between stigma maturity and pollen receptivity in German chamomile, both in self-incompatible and self-compatible genotypes. Therefore, after repeated manual self-pollination, the interactions between pollen and stigma were made visible by staining enzymatic activities and then recorded under the microscope. Accordingly, stigmatic activity of esterases and peroxidases were identified in different floral development stages and compared between self-compatible and self-incompatible genotypes. This study will contribute in characterizing the mechanism of self-incompatibility in German chamomile.

In the first part of the thesis the phenomenon of self-incompatibility is presented. Therefore, the S-locus and the mechanisms of self-incompatibility are outlined. The following part is dedicated to German chamomile, especially to its morphological and generative properties. Then, the enzymes involved in the stigmatic receptivity of German chamomile are explained, namely esterases and peroxidases. In the material and methods section, all information about the implementation of the work steps can be found so that it is comprehensible how the results were collected. This is followed by the presentation of the results, both from the statistical and from the microscopic analysis, which are then discussed in the last part of the thesis.

# 2. Self-incompatibility

Most flowering plants favor out-breeding, cross-fertilization between genetically dissimilar individuals, because it generates and maintains genetic diversity within a species, increasing the chances of survival in a changing environment (Öpik et al., 2005). In contrast, inbreeding and self-compatibility are considered as an evolutionary dead-end, leading to a high species extinction rate (Fuji et al., 2016). Generally, hermaphrodite plant species, such as German chamomile, are able to reproduce through a combination of self-fertilization and outcrossing (Voillemot & Pannell, 2017). A selective advantage achieved by self-fertilization is the assurance of reproduction through the ability to reproduce in the absence of mates or pollinators (Iwano & Takayama, 2012; Voillemot & Pannell, 2017). Furthermore, self-fertilization makes it possible for plants to transmit two copies of their genome to their seed progeny instead of just one, which can result in a significant fitness benefit (Voillemot & Pannell, 2017). On the other hand, self-fertilization is supposed to decrease heterozygosity, leading to the expression of deleterious recessive alleles and an increase in inbreeding depression (Iwano & Takayama, 2012; Voillemot & Pannell, 2017). The effects of inbreeding depression, expressed as the decline in fitness of progeny derived from inbreeding compared to those derived from out-crossing, are considered one of the most important selective forces in the evolution of sexual systems in hermaphrodite populations (Husband & Schemske, 1996; Voillemot & Pannell, 2017) and therefore are used to explain the evolution and maintenance of reproductive systems that enhance cross-fertilization (Husband & Schemske, 1996). An effective mechanism for ensuring cross-fertilization is self-incompatibility (SI), which is found in approximately 40% of flowering plant species and in at least 100 families (Fuji et al., 2016). Angiosperms, especially hermaphrodite plant species, have developed self-incompatibility as a genetic system for the prevention of inbreeding and thus promote outcrossing between different individuals within the population (Iwano & Takayama, 2012). Lundqvist (1964) defined self-incompatibility in higher plants as the inability of a fertile hermaphrodite seed-plant to produce zygotes after self-pollination, what is considered the generally accepted definition in professional circles today (De Nettancourt, 2013). In other words, a flowering plant species is self-incompatible, if it cannot successfully reproduce through self-pollination (Shinozuka et al., 2019). With the one exception of *Borago officinalis*, all known systems of self-incompatibility are pre-zygotic (De Nettancourt, 2013). The need to differentiate between pre- and post-fertilization barriers to selfing is probably academic, nevertheless, in regard to the fundamental differences in function, mechanism and occurrence, it seems recommendable that the term “incompatibility” should not include zygote lethality (De Nettancourt, 2013).

German chamomile is said to be a mainly out-crossing plant species, however, exact values for the proportions of cross-fertilization and self-fertilization are not known (Faehnrich et al., 2013). Within the Asteraceae, SI is very common with about 63% of species possessing the ability of SI (Ferrer & Good-Avila, 2007; Novak et al., 2019), whereas the remaining species exhibit a mixture of pseudo-self-incompatibility (PSI, 10%) and self-compatibility (SC, 27%) (Allen et al., 2011). This high percentage of self-incompatible species in the Asteraceae, indicates that SI may be the ancestral breeding system within the family (Allen et al., 2011).

There are different genetic systems of self-incompatibility, all of which are thought to have evolved repeatedly among flowering plant species (Öpik et al., 2005), but the underlying principle is always the same – if the pollen and the pistil express the same allele of an SI gene (S), the fertilization process is stopped (Pichler, 2016). Accordingly, it can be stated, that the term self-incompatibility is describing a situation, which involves a participation from both the pollen and the pistil (De Nettancourt, 2013), what is considered a fundamental process in the reproductive biology of flowering plants (Allen et al., 2011). These so-called pollen-pistil

interactions are composed of a complex series of cellular and molecular interactions between male and female determinants of the response (Öpik et al., 2005). The key part of these interactions involves a recognition step, which requires the stigma or style to distinguish "self" from "non-self" pollen (Öpik et al., 2005; Shinozuka et al., 2019), often associated with active processes of discrimination and rejection of "incompatible" pollen at interspecific and intraspecific levels, whereas the incompatible pollen is represented by "self-pollen" or pollen from genetically closely related individuals (Allen et al., 2011). Generally, there are two fundamentally different types of events that are considered to constitute the basis of an SI system - on the one hand, there is the stimulation of unlike genotypes and, on the other hand, the inhibition of like genotypes (De Nettancourt, 2013).

## 2.1. The *S*-locus

Classic genetic studies have established that self-incompatibility is under genetic control and, in many species, is regulated by a cluster of tightly linked genes that are located at a single multiallelic locus – the *S*-locus (Öpik et al., 2005; Iwano & Takayama, 2012). This multigene complex at the *S*-locus is inherited as one segregating unit, and hence the variants of the gene complex are referred to as *S*-haplotypes, whilst the variants in the individual genes are referred to as alleles (Öpik et al., 2005; Takayama & Isogai, 2005). Each *S*-haplotype carries both male and female specificity determinants, called the *S*-determinants (Fuji et al., 2016). Various recognition processes take place, associated with specific cellular and molecular interactions between these *S*-determinants, inducing acceptance or rejection of the pollen (De Nettancourt, 1997; Fuji et al., 2016). The molecules involved in this SI recognition are usually encoded by genes of the *S*-locus. Pollen inhibition occurs when the same "*S*-allele" specificity is expressed by both pollen and pistil (Takayama & Isogai, 2005).

Conventionally, genetic homomorphic SI systems have been classified into two types (Fuji et al., 2016). Gametophytic self-incompatibility (GSI) on the one hand and sporophytic self-incompatibility (SSI) on the other hand, based on modes of genetic control of pollen SI phenotype. These two mechanisms are controlled by the *S*-locus and can be distinguished by the site of pollen-pistil interaction (Aslmoshtaghi & Shahsavar, 2016). In SSI, the incompatibility reaction occurs on the surface of the stigma, whereas, in GSI, it is located on the stigma or in the upper region of the style.

## 2.2. Gametophytic self-incompatibility

Gametophytic self-incompatibility systems (GSI) have a common molecular basis across numerous plant families (Koseva et al., 2017). They have already been identified in 60 families (Kao & McCubbin, 1996) and may be the ancestral condition for flowering plants (Koseva et al., 2017). Plant species applying gametophytic SI are usually characterized by a "wet" stigma, which gives the pollen both hold and a nutrient medium on arrival (Wagner, 2016). When a viable pollen grain lands on the stigma surface, it usually germinates and the pollen tube grows into the female tissue of the matrix, the stylus canal. Subsequently, RNA-hydrolyzing enzymes, called RNases (ribonucleases), of the maternal tissue penetrate into the pollen tube (Pichler, 2016). Hereby, a contact between the molecules of the two partners is enabled, resulting in a specific recognition reaction that in turn triggers a signal cascade (Öpik et al., 2005; Wagner, 2016). The recognition of the pollen is controlled by genes or *S*-alleles (Wagner 2016), whereas, in gametophytic SI, only the genotype of the haploid pollen itself determines *S*-specificity (Fuji et al., 2016). If the haploid pollen carries an *S*-allele that matches an *S*-allele of the style, the ribonuclease molecules that migrate into the pollen tube inhibit translation there, causing the pollen tube to stop growing (Öpik et al., 2005; Wagner, 2016). If it carries another *S*-allele, the penetrating RNases are switched off in a way that has not been understood

so far, and the pollen tube can continue to grow (Pichler, 2016). For example, an *S1*-pollen grain from an *S1S2*-sporophyte parent cannot fertilize ovules from an *S1S2*-flower, but it can fertilize ovules from an *S2S3*-flower. An *S2*-pollen grain can fertilize neither of the two flowers.

### 2.3. Sporophytic self-incompatibility

By contrast, sporophytic self-incompatibility systems (SSI) have at least 17 distinct evolutionary origins and occur across 10 plant families (Koseva et al., 2017). Plant species that show SSI are considered to have a "dry" stigma and, therefore, do not offer the pollen a medium for germination (Wagner, 2016).

In sporophytic SI, the genotype of diploid donor tissues determines pollen *S*-specifity. This means, that the pollen response is determined by the diploid genotype of the pollen-forming individual and not by the allele of the pollen grain. Thus, all pollen grains of this plant have the same reaction, even though they have different alleles (Fuji et al., 2016). The allelic interactions are critically important for *S*-specificity determination in SSI - in case of only one accordant allele of maternal and paternal plant, a fertilization is totally inhibited. For example, neither an *S1*- nor an *S2*-pollen grain from an *S1S2*-sporophyte parent can fertilize the ovules of an *S1S2*-flower or *S2S3*-flower (Odenbach, 1997; Faehnrich et al., 2015).

Extensive research of SI systems has been done in many families, such as Brassicaceae, Papaveraceae and Solanaceae, providing insightful knowledge and revealing that SI encompasses a collection of diverse molecular mechanisms within different families even when they share the same genetic basis (Lou, 2018). In the Brassicaceae, the *S*-locus consists of two closely linked genes, the pistil-expressed *S*-locus receptor kinase (SRK) and the male (pollen) determining *S*-locus cystein-rich protein (SCR) (Koseva et al., 2017). In the Asteraceae, SI systems have been studied for example in *Senecio squalidus* (Allen et al., 2011), *Helianthus annuus* (Sharma & Bhatla, 2013) and *Matricaria chamomilla* (Faehnrich et al., 2013; Faehnrich et al., 2015), whereas *Senecio squalidus* (Oxford Ragwort) is taken as an Asteraceae model species to investigate SI (Lou, 2018). Genetic studies have shown that, SI in *Senecio squalidus*, like other Asteraceae species, is controlled sporophytically by a single *S*-locus (Hiscock et al., 2000), however, the genes responsible for SI are different than those in *Brassica* species (Hiscock et al., 2003; Novak et al., 2019), indicating a different mechanistic basis for SSI than the SRK/SCR system of Brassicaceae (Allen et al., 2011; Koseva et al., 2017). Despite the extensive research of SSI in *S. squalidus*, the genetic *S*-locus controlling SSI remains unidentified.

# 3. German chamomile

## 3.1. Systematology

German chamomile (*Matricaria chamomilla* L., syn. *Matricaria recutita* L., syn. *Chamomilla recutita* (L.) Rauschert) is a well-known medicinal plant species from the Asteraceae family (also referred to as Compositae), which is part of the flowering-plant order Asterales.

Even though the systematic status is quite clear today, there are several inaccuracies regarding the nomenclatural situation of German chamomile. The binomial name *Matricaria chamomilla* L. was originally first published in 1753 by the Swedish botanist Carl von Linné (1707 – 1778) in Species Plantarum (ed. 1: 891, Nr. 3). However, the plant he was describing as "*Matricaria chamomilla* L. 1753" is obviously not German chamomile. As a matter of fact, applicable to German chamomile is the name of another species that was published at the same time, i.e. *Matricaria recutita* L. (Franke & Schilcher, 2005). Then again, when referring to the International Rules of Botanical Nomenclature, the legitimate name for German chamomile should be *Chamomilla recutita* (L.) Rauschert. The name of the genus *Matricaria* derives from the Latin "*matrix*", meaning womb, and has most probably been given because German chamomile was widely used to treat various female complaints, such as menstrual cramps and sleep disorders related to premenstrual syndrome. The term "*chamomile*" comes from the Latin "*chamomilla*" and originates from the Greek word "*chamaimelon*". The term "*chamai*" means "on the ground, low, short", whereas "melon" stands for "apple", because the scent resembles the aroma of some apples (Hünemörder, 2006). Not only the naming of numerous pharmaceutical preparations as well as the names of substances such as "*Chamillin*" and "*Chamazulen*" are connected to this term, but also national designations such as "*Kamille*", "*Camomille*", "*Kamilica*", "*Camomile*", "*Kamilky*", "*Chamomila*" and "*Camomilla*" (Toman & Stary, 1965).

As mentioned above, in the first edition of his Species Plantarum Linné made mistakes that he corrected later on, but the consequences still continue to exist today in the form of differences to be found in botanical literature. Nevertheless, in this study, German chamomile will be referred to as *Matricaria chamomilla* L. (see Table 3.1).

**Table 3.1:** Scientific classification of *Matricaria chamomilla* L.

| | |
|---|---|
| Kingdom | Plantae |
| Clade | Tracheophytes |
| Clade | Angiosperms |
| Clade | Eudicots |
| Clade | Asterids |
| Order | Asterales |
| Family | Asteraceae |
| Subfamily | Asteroideae |
| Supertribe | Asterodae |
| Tribe | Anthemideae |
| Genus | *Matricaria* |
| Species | *M. chamomilla (L.)* |
| Binomial name | *Matricaria chamomilla* L. |
| Common name | German chamomile |

## 3.2. General information

German chamomile is an annual, herbaceous plant with fibrous roots and a sweet scent. It has an erect, branched, flexuous and leafy stem, which grows from 10 to 90 cm in height. The double or triple pinnate leaves have a green color and a lanceolate, oval shape. The size of their outline is approximately 1 to 7 cm in length and 1,5 to 2,5 cm in width (Sell & Murrell, 2006). All of the lobes are narrowly linear or almost filiform. The leaf arrangement is semi-amplexicaul and alternate along the stem, with one leaf per node. Both, stem and leaves are glabrous. The fruit is an achene of about 0,8 to 1,5 mm in length, which contains a single seed and lacks a pappus. It appears somewhat compressed, slightly curved like a horn, but is smooth on the back and without oil glands. The seeds are very hardy and may remain dormant in the soil up to 15 years before germination (Albrecht & Otto, 2020).

The flowers of German chamomile, as characteristic for the family Asteraceae, are borne in paniculate flower heads, also referred to as the capitulum. The radial arrangement of the numerous tiny flowers, called florets, causes the capitulum to resemble a single flower. When taking a closer look, however, it becomes clear that it actually consists of an outer circle of 11 to 24 zygomorphic ray florets and an inner circle of 400 to 500 actinomorphic disc florets, all sharing the same receptacle (Albrecht & Otto, 2020). Depending on the floral development stage, the shape of the receptacle develops, starting from being flat and disc-shaped, it grows into a conical form at first. As maturation proceeds, the shape becomes more globular and elongated until the receptacle reaches its final size, being approximately 6 to 8 mm in width. Nonetheless, the structure of the receptacle remains hollow from the beginning onwards. The involucre, which is located below the capitulum, is composed of a group of small, green bracts, also called phyllaries. These involucral bracts have a size of about 1,5 to 3 mm in length and 1 to 1,5 mm in width, arranged in one to three rows, resembling a calyx (Sell & Murrell, 2006).

The disc florets are located in the center of the capitulum. They are perfect flowers, both male and female fertile. They have a size of approximately 1,5 to 4 mm in length and a yellowish orange to pale yellow color. The tubular corolla usually exists of five fused petals, creating a penta-dentate shape at the apex. The disc florets are provided with five syngenesious stamens, meaning that they have free filaments and fused anthers. The ray florets, also referred to as ligulate florets, are situated in the outer rows of the radiate capitulum. They are male sterile and female fertile. As a special feature, the corolla is characterized by an irregular structure. At the base it is tubular, but three of the coalesced petals elongate on the outer side into a generally flat, strap-shaped projection representing the actual ray, also referred to as ligule. The ligules are white in color and grow up to 10 mm (usually 6–8 mm) in length and approximately 2 mm in width (Sell & Murrell, 2006).

The pistil is the female reproductive structure. It is located in the central position of a floret. In German chamomile, the pistil is built up from two carpels which are united to form a compound ovary with a terminal style. The ovary includes one loculus holding a single anatropous ovule with a basal placentation, which implies that the ovule is attached to the base of the ovary. The stamen is the male reproductive structure. It consists of the pollen-producing anther, which is supported by a filament. The styles of German chamomile usually feature a two-branched, in exceptional cases a three-branched stigma at the apex. Furthermore, the styles of German chamomile, as typical for the plant species of Anthemideae, are known to be senecioid (Erbar & Leins, 2015) meaning that they resemble the ones of the genus Senecio. Such senecioid styles are characterized by specialized secretory cells, also referred to as papillae, that compose the stigma epidermis. Typically, these stigmatic papillae cells have striae on their top, and are found in combination with longer stylar hairs, also referred to as pseudo-papillae.

These pseudo-papillae, exhibiting longitudinal cuticular striations, are situated on the apex of the stigma branches and act as “pollen-presenters”.

### 3.3. The pollen

The angiosperm pollen grain consists of three distinct parts. The central part is the internal cellular material, which is the source of nuclei responsible for fertilization. The two other parts form the pollen grain wall, also referred to as sporoderm, which is composed of an outer layer, the exine, and an inner layer, the intine (Kearns & Inouye, 1983). The intine is a soft, barely resistant shell, which is made up from two to three layers, enveloping the vegetative cell. The two inner layers are rich in cellulose, the outer layer mainly consists of pectin. During pollen grain germination on a stigma surface, the intine forms the wall of the pollen tube, growing through the apertures (Faber, 2017). The exine forms the outer layer of the sporoderm and consists mainly of sporopollenin, a very resistant polyester made from fatty acids and carotenoids. It is divided into two layers. Inside, there is the smooth nexine layer, which is laid up on top of the intine. The sexine covers the pollen grain from the outside. Located in the spaces between the tectum, which is a part of the sexine, there is the “Pollenkitt”. It is a sticky mass, which is important for the adherence of the pollen grains in pollen packets and the adherence to pollinators. In addition, incompatibility proteins are stored in the tectum, which prevent self-pollination. The sexine is sculptured, giving the pollen grain its typical morphology (Faber, 2017). At certain points in the sporoderm, the pollen grain does not have an exine layer. These points are called apertures. They are necessary so that the intine is able to grow through the pollen grain wall during the fertilization process, in order to subsequently form the pollen tube (Faber, 2017). Since characteristics such as the exine sculpturing and the size and number of apertures are readily recognizable under the microscope, they proved to be useful as taxonomic tools for classifications in the systematics of the Asteraceae (Shabestari et al., 2013). The viable pollen grains of German chamomile are about 30 µm in size. They have a rounded, triangular shape with short spine exines and three apertures (Pichler, 2016).

Pollen is produced within the anthers of the flowers. During its development, the anther forms two general groups of cells, reproductive cells and non-reproductive ones (Borg & Twell, 2011). The reproductive cells give rise to the microspores. The non-reproductive cells form various anther tissue layers, namely the epidermal, cortical and tapetal cell layers that surround the reproductive cells (Roberts, 2007).

Two different and successive developmental phases result in the production of the mature microgametophytes (Roberts, 2007). During the first phase, the so-called microsporogenesis, the diploid reproductive cells differentiate as pollen mother cells (microsporocytes), which divide by meiosis to form four haploid microspores. Each diploid pollen mother cell creates a tetrad of four haploid microspores, leading to the formation of distinct single-celled haploid microspores. The second phase, the so-called microgametogenesis, is initiated by the expansion of the microspore, usually involving the formation of a single large vacuole (vacuolation) and a displacement of the microspore nucleus towards the microspore wall. The nucleus undergoes first pollen mitosis (PMI) resulting in the production of a bicellular pollen grain, which is composed of a small generative cell enclosed within a large vegetative cell cytoplasm. The two unequal cells each contain a haploid nucleus. Subsequently, the vegetative cell exits the cell cycle, but the enfolded generative cell elongates and divides once more at pollen mitosis II (PMII), giving rise to a pair of sperm cells completely enclosed within the vegetative cell cytoplasm (Rutley & Twell, 2015). This happens either before pollen is shed (tricellular pollen) or within the pollen tube (bicellular pollen). The pollen of German chamomile is shed in the trinucleate stage, meaning that one nucleus constitutes a vegetative nucleus, while the other two function as sperm nuclei. In general, pollen of Asteraceae has a very short life, whereas its

longevity is affected by temperature and humidity. Too high or too low temperatures may inhibit pollen tube growth, which is usually ideal between 25°C and 30°C (Singh & Kao, 1992).

### 3.4. Pollination

Pollination is one of the most critical phases in the life cycle of a flowering plant (McInnis et al., 2006). It is an essential feature of sexual reproduction in angiosperms and commonly defined as the transfer of pollen from anther to stigma (Stoskopf, 1993; Öpik et al., 2005). Generally, the plant species within the Asteraceae family, together with the other families in the order Asterales, are typically characterized by a special pollination mechanism known as "secondary pollen presentation" (Howell, 1993). In simplified terms, secondary pollen presentation can be described as a pollination mechanism in which the pollen grains are not presented directly out of the anthers but, just before or at the onset of anthesis, are transferred from the anthers onto another floral organ, which then functions as the pollen presenting organ for pollination (Howell, 1993; Leins & Erbar, 2006). When relating to the organ used for presentation and how the pollen is loaded onto the presenting surface, German chamomile may be classified as a "terminal stylar presenter with an active pollen placement" (Howell, 1993), meaning that the organ used for presentation of the pollen is represented by the distal portion of the style onto where the pollen is actively loaded. In order to enable a precise loading of the presenting surface, German chamomile, together with other members of Asteraceae, shows a very specific anther morphology and a close association of the presenting organ to the anthers prior to anthesis. The anthers are united by the coherence of their cuticles to form a connate ring, hereinafter referred to as "the anther tube", surrounding the immature style. Growth of the stamen-corolla tube and filaments causes the anther tube to be raised above the stylar tip. After that, the pollen grains are actively loaded into the cavity of the anther tube and onto the style, via the action of introrse dehiscing anthers just before anthesis (Leins & Erbar, 2006; Howell, 1993). The anther dehiscence occurs almost simultaneously, thereby nearly all of the pollen is shed onto the distal portion of the style when the flower opens (Howell, 1993). The distal portion of the style in German chamomile normally features two, in exceptional cases three, truncated stigma branches provided with a lateral ring of more or less long hairs on their apex, the pseudo-papillae. This specific characteristic of the style morphology is very suitable, because as the style elongates, it perfectly acts as a piston pushing the pollen grains out of the anther tube (Erbar & Leins, 2015) and finally presenting the pollen to pollinators, such as animal vectors. An initial auto-pollination is prevented, because, at this developmental stage, the stigmatic branches of the style are still closely appressed, hereby concealing the receptive stigmatic surface. Whereas the unreceptive outer surfaces of the appressed stigmatic branches terminate the style (Howell, 1993). With maturation of the flower the stigmatic branches gradually spread apart, thus making the receptive inner side accessible to the pollen. At this point, the actual pollination may proceed. This can occur by physical means (e.g. wind) or animal vectors. Near the end of the receptive state, the stigmatic branches eventually reflex back with their pollen-covered hairs onto the presenting surface and auto-pollinate with the goal of ensuring seed production by means of self-pollination, though many species are sporophytically self-incompatible (Howell, 1993).

The first important step in pollination of plant species with a dry or semi-dry stigma, is the adhesion of the pollen grain to the papillae surface. Initial adhesion of the pollen grain occurs very quickly. The stigma surface becomes altered at the interface and acquires a pattern which interlocks with the exine (Zinkl et al., 1999). It is assumed, that the molecules of the exine wall might play an important role in this process. After this initial exine-mediated contact, the pollen grain immediately releases the pollen coat from the exine onto the stigma surface called "coat conversion", where it forms a complex structure that resembles an "attachment foot" in the

zone of contact between pollen and stigma. The structure of this attachment foot is composed of a mixture of pollen-wall material and stigmatic extracellular secretion. The papillae cells cover the receptive surface of the stigma. Since the cytoplasm of the papillae cells contains large numbers of vesicles, it is assumed that these cells are actively producing the secretion (Hiscock et al., 2000; Hiscock et al., 2002). Following adhesion to the stigma surface, the pollen grain hydrates and then germinates to produce the pollen tube. The hydration of a pollen grain on a semi-dry stigma, such as the stigma of *Senecio squalidus*, occurs within 15 to 30 minutes after adhesion to the stigma surface (Allen et al., 2011). A mature pollen grain is already equipped with many of the proteins and RNAs that are required for germination and pollen tube growth. For germination, the cytoplasm of the pollen grain grows out of one of the apertures, while pushing the generative cells into the tip. The tip is where the actual growth of the pollen tube occurs from. Behind this tip, there is a dense cytoplasm rich in vesicles. These vehicles unite with the plasma membrane of the pollen tube, thus providing cell-wall material for rapid growth. The wall of the pollen tube consists of two layers, an outer layer which is composed of pectin, hemicellulose and cellulose, and an inner layer which is rich in callose but absent in the area of the tip (Öpik et al., 2011). The esterified pectins provide the apex with sufficient strength and elasticity to support polarized tip growth (Rejon et al., 2012). The pollen tube grows through the attachment foot into the papillae cell wall, where it must first penetrate the stigma surface and successfully overcome the cutin layer. However, studies of pollen tube growth in German chamomile have shown that pollen tubes do not only grow into the papillae directly, but also into the sides of stigma branches (Faehnrich et al., 2015). Nevertheless, the pollen tube has to breach the cutin layer, only then it may grow intercellularly through the cells of the stigmatic cortex (Hiscock et al., 2002). The cytoplasm moves down the pollen tube as it grows, being sealed off behind with callose plugs. The tubes deposit these callose plugs periodically to avoid floating back of the fertilization cells and to separate them from the empty tube behind (Faehnrich et al., 2015). Thereby, the two sperm nuclei and the vegetative nucleus are transferred through the pollen tube down the stylar tract until the tube tip enters the embryo sac via the micropyle (Öpik et al., 2011). After the pollen tube enters the female gametophyte and grows into the synergid, it bursts open to release its contents. In a process known as "double fertilization", one of the two sperm cells within the pollen tube fuses with the egg cell of the ovule, enabling the development of an embryo. The other cell combines with the two subsidiary sexual nuclei of the ovule and initiates the formation of a reserve food tissue, the endosperm. Finally, the growing ovule develops into a seed (Bhojwani et al., 2015).

## 3.5. The stigma

Understanding floral morphology and biology is fundamental to evaluate pollen-pistil interactions (Souza et al., 2016). Physiological, cytochemical, biochemical and structural features of the stigma are significantly important in the sexual life of a plant resulting in effective post-pollination events (Dey et al., 2016).

The stigma is typically enclosed by a lipidic cuticle, which serves as a protective barrier. The structure of the cuticle is formed by lipid and hydrocarbon polymers impregnated with wax, whereas cutin is one of the major cuticle polymers. Cutin is a polyester composed predominantly of hydroxy and epoxy fatty acids with C16 and C18 carbon chains and their derivatives, interlinked by ester bonds, with the specific cutin composition being variable according to the plant species (Pio & Macedo, 2009). During its development, the stigma undergoes several structural and biochemical changes. At the time of maturity, the receptive stigma surface usually provides diverse biomolecules including carbohydrates, proteins, lipids, enzymes, elements like calcium and boron, flavonoids and reactive oxygen species (Sharma, 2017). The various biochemical components of the stigma enable the recognition of compatible

pollen. Upon pollination, multiple cell to cell signaling events between the female sporophyte and the male sporophyte (pollen) take place resulting in the identification of pollen (Sharma, 2017).

In order for pollination to occur, not only must viable pollen be transferred to the stigma, but it must also be transferred during the period of stigma receptivity (Kearns & Inouye, 1993). Stigma receptivity can be described as the ability of the stigma to capture pollen by adhesion, to let it hydrate and consequently to support pollen germination and tube growth of viable, compatible pollen grain (Dey et al., 2016; Sharma, 2017). If the stigma is not in a receptive state, the pollen may not adhere or may not germinate (Kearns & Inouye, 1993). Stigma receptivity occurs only for a short period during the lifetime of a flower, varying from minutes to a few days, whereas the receptive phase can occur in different phases of flower development (Souza et al., 2016). The presence of several enzymes was found to coincide with this developmental stage (Dafni & Maues, 1998). Observation of the activity of these enzymes can be used as a tool to characterize stigma receptivity. In general, the receptivity is maximal shortly after the start of the anthesis, which is also reflected in a high enzymatic activity, but there may be significant differences from species to species (Dey et al., 2016).

Classically, angiosperm stigmas can be divided into two basic categories, wet and dry, depending on the absence or presence of exudates on the stigma surface at the time of maturity (Heslop-Harrison & Shivanna, 1977; Heslop-Harrison, 1981).

The epidermal cells (stigmatic papillae) of wet stigmas possess a surface characterized by areas of disrupted cuticula (Hiscock & Allen, 2008). At maturity, they produce a copious surface secretion which may be either mainly lipidic, as for example in Solanaceae, or mainly aqueous, as found in Liliaceae (McInnis et al., 2006). The secretion contains lipids, proteins, carbohydrates, phenols, glycoproteins, ions and enzymes, such as esterases and peroxidases (Sharma & Bhatla, 2013). Pollen adhesion is enabled by the stickiness and surface tension of the stigmatic secretion (Réjon et al., 2012), whereas pollen capture is non-specific (Allen et al., 2011). Pollen hydration on wet stigmas is an unregulated process and occurs passively within the secretion. The penetration of the stigma by the pollen tube is relatively easy, due to the lack of a continuous cuticle (Allen et al., 2011).

In contrast, the epidermal cells (stigmatic papillae) of dry stigmas are surrounded by a continuous layer of cutin (Hiscock & Allen, 2008) and there is no production of a surface secretion. Instead, extracellular components are present in form of a thin, extracuticular hydrated layer pellicle (Dey et al., 2016), referred to as the proteinaceous pellicle (McInnis et al., 2006). The major components of the pellicle are glycoproteins, carbohydrates, lipids and some enzymes, predominantly esterases and peroxidases. At the time of maturity, it is produced within the stigmatic papillae and appears on the stigma surface via discontinuities in the cuticle (Sharma, 2017). Interaction of this proteinaceous pellicle together with the pollen coat enables pollen adhesion (Réjon et al., 2012), whereas pollen capture and adhesion show a degree of species specificity (Allen et al., 2011). Pollen hydration on a dry stigma is a highly regulated process, and the continuous cuticle presents a major barrier to pollen tube penetration. This barrier must be overcome by pollen secreting hydrolytic enzymes, such as cutinases (Allen et al., 2011).

Differences between the “wet” and “dry” classes of stigma intergrade (Heslop-Harrison & Shivanna, 1977). Early studies claimed that Asteraceae species have the dry-type stigma (Vithanage & Know, 1977). However, later studies have revealed that they are not entirely dry, but rather show characteristics of both dry and wet stigma surfaces (Hiscock et al., 2002). Especially, studies on the stigma of *Senecio squalidus* have shown that the stigmatic papillae cells are covered by a cuticle which is not continuous at the base. Furthermore, it was shown

that, at the time of maturity, small amounts of secretions containing lipids, carbohydrates and proteins, are produced in the basal regions of stigmatic papillae where the cuticle is absent, and, if there is contact with a pollen grain, secretion is even increased, regardless of whether the pollen is compatible or incompatible (Hiscock et al., 2002). Consequently, the stigma of Asteraceae species has been reclassified as “semi-dry”. In spite of all, so far, the stigma type of German chamomile cannot be clearly determined, since there is no precise data on the presence of a disturbed cuticle in the area of the papillae.

# 4. Enzymes involved in pollen-pistil interactions

## 4.1. Esterases

The reproductive success of German chamomile is significantly determined by pollen-stigma interactions, including pollen adhesion, pollen recognition, pollen hydration, pollen germination, growth of the pollen tubes, pollen tube entry into the stigma and the incompatibility response (Sharma & Bhatla, 2013; Sharma, 2017). The components of pollen coat, exine and pellicle play an important role in these processes. With the onset of stigma receptivity, a secretory activity starts in the papillae resulting in the accumulation of extracellular secretions. Thus, contained within the secretions, proteins are located on the papillae surface. These stigma surface proteins interact with pollen coat proteins, which enables the process of adhesion. The pollen coat proteins are considered to be important in discrimination and recognition of pollen grains. The subsequent process of pollen hydration is complex and depends on the proteinaceous and lipidic components of pollen and stigma (Sharma & Bhatla, 2013). In order to breach the outside barrier of the stigma, serine esterases present in the pollen coat, along with the esterases on the stigma surface, form a cutinase complex which causes the breakdown of the cuticula (Hiscock et al., 2002; Rejon et al., 2016). Cutinases (EC 3.1.1.74) are known to catalyze the hydrolysis of polyesters in the cuticle of plants (Nyyssölä et al., 2015). They are a group of extracellular enzymes that belong to the carboxylic-ester hydrolases. Carboxylic-ester hydrolases (EC 3.1.1.-) are esterases (EC 3.1) acting on carboxylic ester bonds (Novak et al., 2019). They catalyze the hydrolysis of carboxylic acid esters, leading to the formation of an alcohol and a carboxylic acid anion (Rejon et al., 2012). Cutinases are classified as serine esterases which possess the classical Ser-His-Asp triad, sharing catalytic properties of several lipases and esterases (Pio & Macedo, 2009; Nyyssölä et al., 2015). They are widely distributed in nature. Typically, plant pathogenic fungi overcome the cuticle of higher plants using cutinases (Nyyssölä et al., 2015; Novak et al., 2019). Furthermore, also bacterial cutinases have been discovered. Both bacterial and fungal cutinases are assumed to function by degrading the plant cuticle during infection of a plant (Takahashi et al., 2010). In fact, there are numerous enzymes in a mature plant pollen grain, many of which are released from the pollen coat to the extracellular space immediately when it attaches to the stigma surface (Rejon et al., 2012; Rejon et al, 2016). Enzymes belonging to four esterase subgroups were so far identified in pollen from different species, these include, acetylcholine esterases (EC 3.1.1.6), cholinesterases (EC 3.1.1.7), pectine esterases (EC 3.1.1.11), and several active cutinases [EC 3.1.1.74]. In particular, cutinases and pectine esterases are significantly involved in pollen-pistil interaction (Novak et al., 2019). Cutinases (EC 3.1.1.74) break down the waxy polymers of cutin present in the stigma cuticle and hence are indispensable for pollen tubes when penetrating through the cutin layer (Rejon et al., 2012; Novak et al., 2019). Pollen pectin methylesterases (EC 3.1.1.11) are involved in the regulation of pollen tube wall dynamics and thus could play an important role for the intercellular tube growth within pistil tissues (Rejon et al., 2012; Novak et al., 2019). Furthermore, various lipases (EC 3.1.1.3) that occur in the pollen coat of *Arabidopsis thaliana* and *Helianthus annuus* are assumed to play a part in the decomposition of lipidic structures, such as the cuticle (Rejon et al., 2012). There are many other pollen esterases, but the identity and function of most are still unknown.

## 4.2. Peroxidases

During pollen–stigma interactions in dry and semi-dry stigmas, the first layer that comes in contact with pollen grains is the thin pellicle which overlays the cuticula (McInnis et al., 2006; Sharma & Bhatla, 2013). Peroxidases (EC.1.11.1.x) represent one of the key antioxidant

enzymes that are associated with the pellicle. They are enzymes that decompose hydrogen peroxide (Pandey et al., 2017). It has long been known, that angiosperm stigmas show a high activity of peroxidases when they are receptive to pollen, nevertheless the biological function of stigma peroxidases has not yet been clearly determined. So far, at least five peroxidases have been identified in the stigma of *Senecio squalidus* (McInnis et al., 2006). Furthermore, in order to explore the function of stigma peroxidases, the level of reactive oxygen species in stigmas of *Senecio squalidus* has been investigated. Significant quantities of reactive oxygen species, mainly hydrogen peroxide ($H_2O_2$), were found, while most of it was produced in the stigmatic papillae, which is the location of stigma specific peroxidases. It has been shown that peroxidases in the stigmatic papillae are associated predominantly with the cytoplasm and the surface of the papilla cell wall. More specifically, stigma specific peroxidases can be found in the proteinaceous pellicle and, they are mainly active at acidic pH, with an optimum level of pH 4,5. However, stigma peroxidases are assumed to have a unique, as yet undefined, biological function within the stigma. Due to their precise papilla-specific expression, they are thought to play a key role in stigma function. The different possible functions of stigma peroxidases include some aspects of pollen-stigma interactions, for instance in weakening stigma cell wall components in order to allow penetration and growth of pollen tubes within the stigma. Another possible function may be an involvement in signaling systems that are activated during the pollen-stigma interaction, such as species-specific pollen recognition. Last but not least, stigmatic peroxidases may contribute to an enhanced protection against microbial attack when the pistil is "primed" to receive pollen (McInnis et al., 2006; Hasanuzzaman et al., 2018). Because peroxidases of unknown function are a universal feature of mature angiosperm stigmas, it makes peroxidase assays a useful tool of assessing stigma receptivity.

### 4.3. Chemical tests for detection of enzymatic activity

Despite the fundamental differences between wet and dry stigma types, they both exhibit high levels of esterase and peroxidase activity when they reach maturity. This indicates that, the accumulation of high levels of esterases and peroxidases is a general feature of angiosperm stigmas when they are optimally receptive to compatible pollen (McInnis et al., 2006; Dey et al., 2016). For this reason, esterases and peroxidases can be used as markers for the degree of stigma receptivity (Rejon et al., 2012). Various chemical tests have been developed to determine the stigma receptivity in vitro. Most of the tests involve enzymatic reactions, under the assumption that enzyme presence reflects stigma receptivity. The method used may vary according to the plant species (Kearns & Inouye, 1993; Dafni & Maues, 1998; Souza et al., 2016). To test for peroxidase enzymes, usually a freshly made benzidine solution is being used. The stain works via oxidation and the subsequent color change of the benzidine, as hydrogen peroxide is broken down by the presence of peroxidase (Kearns & Inouye, 1993). Stigmatic esterase activity is another frequently used enzymatic indicator of receptivity (Kearns & Inouye, 1993). A generally accepted method for the detection of esterase activity is the use of α-naphthyl acetate as a substrate, with fast blue B salt in a coupling reaction. First, the naphthyl alkyl group is liberated by the esterase, then it couples with the dye (Kearns & Inouye, 1993). Once stigma receptivity has been investigated by using one of these techniques, it may be possible to make associations with some developmental change (Kearns & Inouye, 1993).

# 5. Material and methods

## 5.1. Plant materials and growth conditions

The experiments were carried out at the Institute of Animal Nutrition and Functional Plant Compounds of the University of Veterinary Medicine in Vienna, Austria (48° 15’ 25’’ N, 16° 25’ 53’’ E, 161 m). German chamomile plants from the two diploid cultivars “Degumille” and “Bona” (see Table 5.1) were germinated and grown under greenhouse conditions. Cuttings from five genotypes, stable in SI (two from cultivars Bona and three from cultivars Degumille) and cuttings from six self-compatible genotypes (four from cultivars Bona and two from cultivars Degumille) were grown and maintained vegetatively in a growth chamber under long-day conditions in order to induce flowering (16 h/d, 25°C/20°C day/night temperatures, 60 % relative humidity).

**Table 5.1:** List of cultivars

| Cultivar | Ploidy | Year | Country | Parental Population |
|---|---|---|---|---|
| Bona | 2x | 1984 | Slovak Republic | (Spanish origin x Bohemia) * polyploidization |
| Degumille | 2x | 1977 | Germany | unknown |

Tested cultivars of M. chamomilla (L.) with origin and ploidy level (modified after Albrecht & Otto 2020)

## 5.2. Test series

This work includes three different test series (see Table 5.2). The first is defined as "Esterase assay without isolation", or "*ES0*" for short. The second series of tests is called "Esterase assay with isolation", abbreviated as "*ES1*". The third and last of the test series is defined as "Peroxidase assay without isolation", with the abbreviation "*PE0*". As the name suggests, the *ES1* test series is the only one for which the flower heads have been isolated. Furthermore, the stigmas of the *ES0* and *ES1* test series were examined for esterase activity, but those of *PE0* for peroxidase activity.

**Table 5.2:** List of test series

| Test series | Name | Abbreviation |
|---|---|---|
| 1 | Esterase assay without isolation | ES0 |
| 2 | Esterase assay with isolation | ES1 |
| 3 | Peroxidase assay without isolation | PE0 |

Table 5.3 lists the individual genotypes, according to the mode of compatibility and the test series in which they were used. In the test series *ES0*, four different self-compatible genotypes were tested, Bona5, Bona15, Degumille27 and Degumille97, and four self-incompatible genotypes, Bona7, Bona12, Degumille8 and Degumille9. In the test series *ES1*, the four self-compatible genotypes Bona5, Bona19, Bona22 and Degumille27 as well as the four self-compatible genotypes Bona7, Bona12, Degumille9 and Degumille44 were tested. For the test series *PE0*, the three self-compatible genotypes Bona5, Bona15 and Degumille27 as well as the four incompatible genotypes Bona7, Bona12, Degumille8 and Degumille9 were tested.

**Table 5.3:** List of tested genotypes

| Genotype | Compatibility | Test series |
|---|---|---|
| Bona5 | SC | ES0, ES1, PE0 |
| Bona15 | SC | ES0, PE0 |
| Bona19 | SC | ES1 |
| Bona22 | SC | ES1 |
| Degumille27 | SC | ES0, ES1, PE0 |
| Degumille97 | SC | ES0 |
| Bona7 | SI | ES0, ES1, PE0 |
| Bona12 | SI | ES0, ES1, PE0 |
| Degumille8 | SI | ES0, PE0 |
| Degumille9 | SI | ES0, ES1, PE0 |
| Degumille44 | SI | ES1 |

Self-compatible (SC) and self-incompatible (SI) genotypes of the three different test series (*ES0, ES1, PE0*).

## 5.3. Isolation of flower heads

Before the onset of anthesis, several flowers of each plant were isolated with a simple paper bag to protect them from cross-pollination. Therefore, the individual flowers were initially marked with gummed labels which were wrapped around the pedicle and overlapping back on themselves. Subsequently, paper bags were put over the flowers, and a piece of foam was wrapped around the respective pedicles and both then fixed together with a wire.

## 5.4. Manual pollination

With the beginning of anthesis at least three isolated flower heads of each plant were pollinated with each other by hand three times at intervals of two days. For the manual pollination, each plant was separated, and the paper bags were carefully removed from the flowers. All three flower heads were rubbed at each other moving in circles, one after the other and subsequently the paper bags were put back over the flowers.

## 5.5. Determination of floral development stages

Enzymatic activity was determined at three different floral development stages (see Table 5.4). Note that the division of floral development into three stages was arbitrary and made in order to obtain a more holistic picture of the enzymatic activity. Therefore, the division into these three stages shall be made more comprehensible by the following description.

**Table 5.4:** Classification of floral development stages

| Stage | Name | Abbreviation |
|---|---|---|
| 1 | Floral development stage 1 | FD1 |
| 2 | Floral development stage 2 | FD2 |
| 3 | Floral development stage 3 | FD3 |

In the first stage, defined as “floral development stage 1” (FD1), the capitulum has just completed the bud stage and is characterized by a conical shape. The ray florets have become erect and grow in width. Since the vast majority of disc florets are still unopened, the capitulum has a predominantly green appearance. However, a few of the florets slowly begin to open and to turn yellow. This developmental process takes place in a centripetal way, starting with the florets of the outer rings, and progressing spirally towards the centre. During the second stage, defined as “floral development stage 2” (FD2), the capitulum is turning completely yellow and appears to have a globular shape. The ray florets are fully grown and lie down evenly. The majority of the florets have opened, and the staminal tub enveloping the stigma appears above the corolla of each floret. Most of the florets show their stigma, which partly protrudes from

the staminal tub. At this stage, it is already densely covered with pollen. Since the disc florets in the middle of the capitulum have not yet matured, it causes a flat top of the capitulum. In the third stage, defined as “floral development stage 3” (FD3), the capitulum has reached its final size. The last disc flowers have matured, so the capitulum no longer has a flat, but a completely spherical top. The stigmas protrude further from the staminal tub. They are fully developed and covered with pollen. The capitulum is colored completely yellow, while the outer florets that have matured the longest already appear brownish. The ray florets are only loosely attached to the capitulum and begin to fall off, just like the other overripe florets. Figure 5.1 shows flower heads of German chamomile from the three stages of floral development.

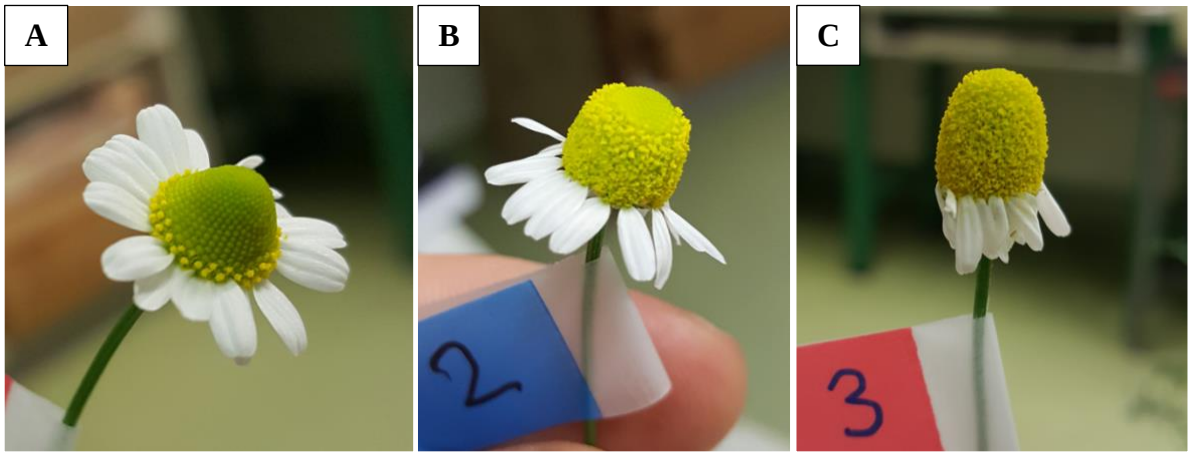


**Figure 5.1:** Flower heads of German chamomile in different stages of floral development. (A) Floral development stage 1. (B) Floral development stage 2. (C) Floral development stage 3.

## 5.6. Isolation of pistils

The first step in assessment of stigma receptivity was to collect and prepare pistils for the further procedures. Therefore, several disc florets were removed from intact chamomile flower heads at the respective stage of development. The single pistils were separated by carefully removing stamens and corolla tubes using dissecting needles (see Figure 5.2). Because the pistils are very susceptible, they were used for further tests immediately after being isolated.

**Figure 5.2:** Isolation of pistils. (Left) Dissecting needles and glass bowls. (Right) Disc floret of German chamomile separated from the flower head, under light microscope (right). The pistil was taken from the disc floret. The glass bowls served as a vessel for the test solutions.

## 5.7. Histochemical detection of enzymatic activity

Two histochemical methods were used to assess stigma receptivity, as described below:

**Esterase Assay:** The first test to identify stigma receptivity was performed by detection of esterase activity with the method described in Serrano and Olmedilla (2012). Briefly, the excised pistils were immersed in a one-to-one mixture of 0,1 % (w/v) 2-naphthyl acetate and 0,2 % (w/v) fast blue B salt in 0,1 M sodium phosphate buffer (pH 7.4) for 1h. Afterwards the pistils were carefully placed on microscope slides in purified water and inspected under a light microscope at 16x, 20x, 40x, 60x magnification (Nikon) to locate the stained areas.

**Peroxidase Assay:** The second method to detect stigma receptivity was performed using the procedure described by McInnis et al. (2006). In this case, peroxidase activity was localized by immersing excised pistils in a solution containing 0,1 M guaiacol, 0,1 M $H_2O_2$, in 20 mM phosphate buffer (pH 4.5), until an orange/red colour was observed (approximately 1–3 min). Hydrogen peroxide ($H_20_2$) localization was performed by immersing excised pistils in a solution containing the ROS (reactive oxygen species) indicator dye TMB (3,3´,5,5´-tetramethylbenzidine-HCl, 0,1 mg $ml^{-1}$ in 0,05 M phosphate buffer, pH 4.5) until a darker color was observed. Again, the pistils were carefully placed on microscope slides in purified water and inspected under a light microscope at 16x, 20x, 40x, 60x magnification (Nikon) to locate the stained areas.

## 5.8. Assessment of enzymatic activity

In order to assess the enzymatic activity, the stigmas were divided into different areas. This subdivision of the stigma was found to be helpful, since often only partial areas were stained during the assays. Figure 5.3 shows a stigma of German chamomile at stage "FD3" which has undergone the esterase assay. It serves to illustrate how each stigma was divided into different sub-areas. The two branches of the stigma were each assessed individually, with the single branches being divided into papillae and pseudo-papillae. In addition, the filament was also assessed individually. Depending on the extent of the staining, degrees of receptivity were assigned: (0) no reaction; (1) very low positive reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

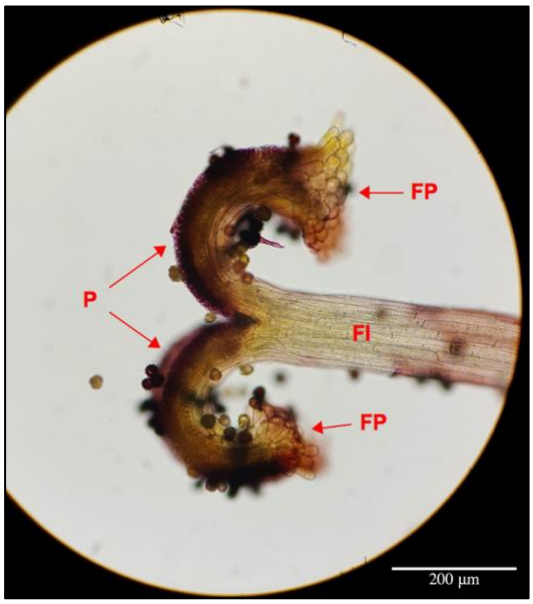


**Figure 5.3:** Detection of enzymatic activity. A stigma of German chamomile (at stage FD3) observed under light microscope (Nikon) at 20x magnitude, showing the different sub-areas that were evaluated to detect the enzymatic activity. Papillae (P), pseudo-papillae (FP) and filament (FI). Bar = 200 µm.

## 5.9. Statistics

Statistical analyses were conducted using the program R (version 3.6.2). First, one-way analysis of variance was performed to determine whether there were significant differences both with regard to the enzyme activities between and within the respective factor levels. Then, post-hoc comparisons were used to determine between which of the factor levels significant differences occurred and whether these were high or low. The first factor was the floral development stage, which was composed of the factor levels *FD1*, *FD2* and *FD3*. The second factor was the compatibility mechanism, with the factor levels *self-compatible* and *self-incompatible*. The third factor was the genotype. Here the factor levels were represented by the *individual genotypes*. The population of statistical units was made up of *papillae*, *pseudo-papillae* and *filaments*. In order to calculate the post hoc comparisons, Tukey's HSD (honestly significant difference) test was carried out. The level of significance was set at 5 %. This means that a significant difference was assumed if the p-value was less than 5 % or $p < 0{,}05$. A value of exactly 5 % or more would accordingly mean that the result was not significant. In addition, differences were classified as highly significant if the p-value was less than 1 % or $p < 0{,}01$.

# 6. Results

The statistical calculations were based on the respective assessment lists, namely those for test series *ES0* (see Appendix A.1, Table A.1), for test series *ES1* (see Appendix A.2, Table A.2) and test series *PE0* (see Appendix A.3, Table A.3).

## 6.1. Results of test series *ES0*

First, the data from the evaluation of the test series *ES0* were subjected to an analysis of variance (see Appendix B.1, Table B.1). Subsequently, post-hoc comparisons were conducted in order to determine significant differences in the mean esterase activity with regard to the individual factors (see Appendix C.1, Tables C.1–C.15).

### 6.1.1. Effect of the "floral development stage" factor

When considering the mean esterase activity (MEA) of all genotypes, generally a weak positive to medium positive reaction was shown for the three stages of floral development (see Table 6.1). As indicated by the results of variance analysis (see Table B.1), the "floral development stage" factor had a significant effect on the stigmatic esterase activity. This was consistent with the results of Tukey's HSD test (see Table C.1).

**Table 6.1:** Esterase activity in different stages of floral development (*ES0*)

| Stage | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| FD1 | 2.207 (50) | 2.446 (50) | 1.492 (49) |
| FD2 | 2.099 (34) | 1.237 (34) | 2.780 (34) |
| FD3 | 3.105 (36) | 2.212 (36) | 2.659 (37) |

Mean values for esterase activity of papillae, pseudo-papillae and filaments in the three stages of floral development (FD1, FD2, FD3). The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

Accordingly, the MEA of the papillae at stage FD1 ($M$ = 2,2) was found to be slightly higher than at stage FD2 ($M = 2,1$) but the differences were not significant ($diff = 0,04$; $p > 0,05$). Eventually, MEA peaked at stage FD3 ($M = 3,1$), whereas both the differences to stage FD2 ($diff = 0,99$; $p = 0,027$) and the differences to stage FD1 ($diff = 0,96$; $p = 0,019$) were found to be statistically significant.

As for the pseudo-papillae, MEA at stage FD1 ($M = 2,5$) was higher than at stage FD2 ($M = 1,2$), while the differences were found to be highly significant ($diff = 1,22$; $p < 0,001$). Finally, MEA at stage FD3 ($2,2$) increased again and remained between that of the other two stages. As well, the differences between MEA at stage FD2 and stage FD3 were identified as statistically highly significant ($diff = 1,06$; $p = 0,0043$). In contrast, the differences between MEA at stage FD1 and stage FD3 were not statistically significant ($diff = 0,17$; $p > 0,05$).

With regard to the filaments, MEA increased between stage FD1 ($M = 1,5$) and stage FD2 ($M = 2,8$), while the differences were found to be highly significant ($diff = 1,42$; $p = 0,0029$). Then, MEA at stage FD3 ($M = 2,7$) slightly decreased again, but the differences to stage FD2 were not significant ($diff = 0,094$; $p > 0,05$). The differences between MEA at stage FD1 and stage FD3, on the other hand, were identified as statistically highly significant ($diff = 1,32$; $p = 0,0046$).

Additionally, the effect of the "floral development stage" factor on the esterase activity of papillae, pseudo-papillae and filaments is graphically illustrated using Figure 6.1.

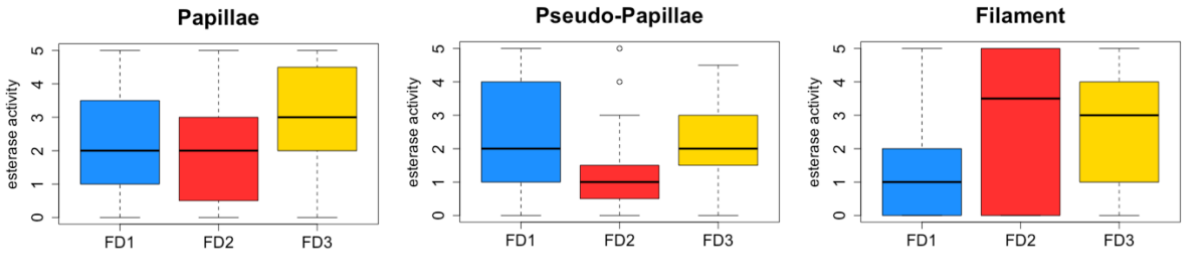


**Figure 6.1:** Variability in esterase activity during floral development (*ES0*). Esterase activity of papillae (left), pseudo-papillae (middle) and filaments (right) in different floral development stages (FD1, FD2, FD3)

### 6.1.2. Effect of the "compatibility" factor

By means of variance analysis (see Table B.1) it can be seen that the factor "compatibility" had no significant effect on the stigmatic esterase activity. In accordance with this result, Tukey's HSD test (see Table C.2) showed that mean esterase activity was almost identical for the self-compatible and self-incompatible genotypes and that there were no significant differences between the two groups. As can be seen in Table 6.2, only the papillae showed slight differences between the MEA of SC genotypes (*M = 2,3*) and that of SI genotypes (*M = 2,6; diff = 0,*31). However, regarding the pseudo-papillae, the MEA of SC genotypes (*M = 2,06*) was almost identical to that of SI genotypes (*M = 2,01; diff = -0,05*), as well as with respect to the filaments, the MEA of SC genotypes (*M = 2,21*) was almost equal to that of SI genotypes (*M = 2,23; diff = 0,02*).

**Table 6.2:** Esterase activity of self-compatible and self-incompatible genotypes (*ES0*)

| Compatibility | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| SC | 2.284 (58) | 2.060 (58) | 2.207 (58) |
| SI | 2.597 (62) | 2.008 (62) | 2.226 (62) |

Mean values for esterase activity of papillae, pseudo-papillae and filaments grouped into self-compatible (SC) and self-incompatible (SI) genotypes. The respective replications are given in brackets.

Furthermore, the effect of the "compatibility" factor on the esterase activity of the papillae, pseudo-papillae and filaments is graphically illustrated by Figure 6.2.

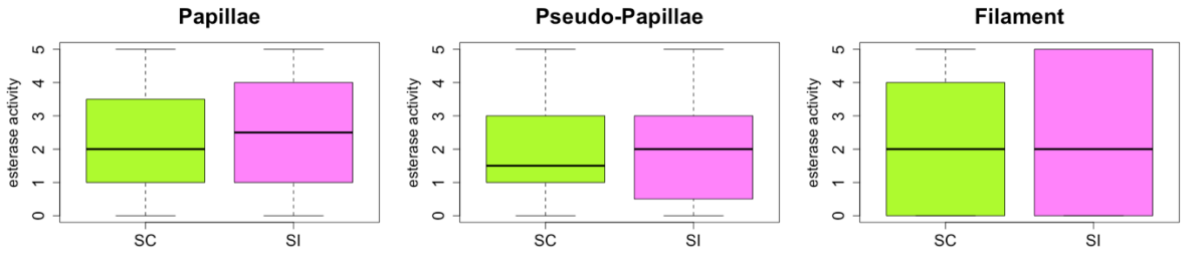


**Figure 6.2:** Variability in esterase activity of self-compatible and self-incompatible genotypes (*ES0*). Esterase activity of papillae (left), pseudo-papillae (middle) and filaments (right) grouped into self-compatible (SC) and self-incompatible (SI) genotypes.

### 6.1.3. Effect of the "compatibility and floral development stage" factor

The results of variance analysis (see Table B.1) show that the "compatibility and stage" factor had no significant effect on the esterase activity of the papillae and pseudo-papillae ($p > 0.05$), while there was a significant influence ($p = 0.041$) indicated for the esterase activity of the filaments, but this was not confirmed by the results of Tukey's HSD test (see Table C.3). The

results of the Tukey HSD test revealed that there were no statistically significant differences ($p > 0,05$) between mean esterase activity of self-compatible and self-incompatible genotypes within the same stages.

Table 6.3 displays mean esterase activity (MEA) at the three stages of floral development with distinction between self-compatible (SC) and self-incompatible (SI) genotypes. The values indicate that in all three stages the papillae of the SC genotypes showed a lower esterase activity than the papillae of the SI genotypes. For the pseudo-papillae and filaments, this was only the case in stage FD1. In stages FD2 and FD3, however, the esterase activity of the SC genotypes was comparatively higher.

**Table 6.3:** Esterase activity of SC and SI genotypes in different stages of floral development (*ES0*)

| **Papillae** | | |
|---|---|---|
| Stage | SC | SI |
| FD1 | 1.979 (23) | 2.415 (27) |
| FD2 | 1.981 (18) | 2.200 (16) |
| FD3 | 3.019 (17) | 3.189 (19) |
| **Pseudo-Papillae** | | |
| Stage | SC | SI |
| FD1 | 2.293 (23) | 2.574 (27) |
| FD2 | 1.296 (18) | 1.176 (16) |
| FD3 | 2.555 (17) | 1.904 (19) |
| **Filament** | | |
| Stage | SC | SI |
| FD1 | 0.967 (23) | 1.957 (26) |
| FD2 | 2.916 (18) | 2.625 (16) |
| FD3 | 3.134 (17) | 2.256 (20) |

Mean values for the esterase activity of papillae, pseudo-papillae and filaments of self-compatible (SC) and self-incompatible (SI) genotypes in different floral development stages (FD1, FD2, FD3). The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

The SC genotypes always showed an increase in esterase activity both between stages FD2 and FD3 and between stages FD1 and FD3. The MEA of papillae was lowest in stage FD1 (*M = 1,98*), remained at the same level in stage FD2 (*M = 1,98*) and reached its peak in stage FD3 (*M = 3,02*). As for the pseudo-papillae, there was a decrease in esterase activity between stages FD1 and FD2. Hence the MEA of pseudo-papillae was lowest in stage FD2 (*M = 1,3*) and highest in stage FD3 (*M = 2,56*), while in stage FD1 it was between the two (*M = 2,29*). The MEA of filaments was lowest in stage FD1 (*M = 0,97*) and then increased continuously over stage FD2 (*M = 2,92*) and stage FD3 (*M = 3,13*).

The SI genotypes showed a decrease in esterase activity between stages FD1 and FD2, followed by an increase between stages FD2 and FD3. This applies to the papillae and pseudo-papillae but not to the filaments, which showed an opposite development of the esterase activity. The filaments first showed an increase between stages FD1 and FD2, and then a decrease between stages FD2 and FD3. While the MEA of papillae at stage FD1 (*M = 2,42*) was already higher than at stage FD2 (*M = 2,2*), it finally peaked at stage FD3 (*M = 3,19*). On the other hand, the MEA of pseudo-papillae was highest at stage FD1 (*M = 2,57*), dropped to the lowest level at stage FD2 (*M = 1,18*) and increased again at stage FD3 (*M = 1,9*). As for the filaments, MEA was initially at the lowest level in stage FD1 (*M = 1,96*), while in the subsequent stage FD2 (*M = 2,63*) it increased to the highest level and then finally decreased again at stage FD3 (*M = 2,26*).

### 6.1.4. Effect of the "genotype" factor

The mean esterase activity (MEA) of the individual genotypes can be seen in Table 6.4. In terms of the self-compatible genotypes, Bona15 showed the highest MEA of papillae (*M = 3,38*) and pseudo-papillae (*M = 2,56*), whereas Degumille97 showed the highest MEA of filaments (*M = 2,92*). In contrast, genotype Bona5 was found to have the lowest MEA of the papillae (*M = 1,69*), the pseudo-papillae (*M = 1,73*) and the filaments (*M = 0,92*).

With regard to the self-incompatible genotypes, Degumille9 had the highest MEA of the papillae (*M = 3,14*), the pseudo-papillae (*M = 2,6*) and the filaments (*M = 3,77*). On the other hand, Bona7 showed the lowest MEA of papillae (*M = 2,41*), while it was almost identical to that of genotype Degumille8 (*M = 2,46*) and genotype Bona12 (*M = 2,44*). Bona12 was also found to be the genotype with the lowest MEA in terms of pseudo-papillae (*M = 1,06*) and filaments (*M = 1,39*).

**Table 6.4:** Esterase activity of the individual genotypes (*ES0*)

| Genotype | Compatibility | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|---|
| Bona15 | SC | 3.382 (17) | 2.559 (17) | 2.176 (17) |
| Bona5 | SC | 1.692 (13) | 1.731 (13) | 0.923 (13) |
| Degumille27 | SC | 1.750 (16) | 1.844 (16) | 2.750 (16) |
| Degumille97 | SC | 2.083 (12) | 2.000 (12) | 2.917 (12) |
| Bona12 | SI | 2.441 (17) | 1.059 (17) | 1.389 (18) |
| Bona7 | SI | 2.412 (17) | 2.294 (17) | 2.353 (17) |
| Degumille8 | SI | 2.464 (14) | 2.214 (14) | 1.714 (14) |
| Degumille9 | SI | 3.143 (14) | 2.607 (14) | 3.769 (13) |

Mean values for esterase activity of papillae, pseudo-papillae and filaments of all self-compatible (SC) and self-incompatible (SI). The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

As indicated by the results of variance analysis (see Table B.1), the "genotype" factor was found to have a statistically highly significant effect on the esterase activity of the papillae, pseudo-papillae and filaments. Tukey's HSD test confirmed this result with the exception of the papillae, for which only statistically significant differences were found (see Table C.4).

Accordingly, the MEA of papillae between genotypes Bona5 and Bona15 (diff = 1,71; p = 0,024) as well as between genotypes Degumille27 and Bona15 (diff = 1,59; p = 0,028) were found to be statistically significant. As for the pseudo-papillae (see Table C.5), the differences between genotypes Degumille9 and Bona12 (*diff = 1,49; p = 0,015*) were found to be statistically significant, while those between genotypes Bona15 and Bona12 were indicated as statistically highly significant (*diff = 1,51; p = 0,0068*). With regard to the filaments (see Table C.6), the differences between genotypes Degumille9 and Bona12 (*diff = 2,28; p = 0,0098*) as well as between genotypes Degumille9 and Bona5 were found to be statistically highly significant (*diff = 2,64; p = 0,0039*). All other comparisons showed no further significant differences.

### 6.1.5. Effect of the "genotype and floral development stage" factor

As can be seen in Table 6.5, the level of mean esterase activity (MEA) was found to be most intense in the self-compatible genotype Bona15 as well as in the self-incompatible genotype Degumille9. Regardless of the compatibility mechanism, there was generally always an increase in mean esterase activity of the papillae between stage FD2 and stage FD3. The same applies to the pseudo-papillae with the exception of genotype Degumille8.

As for the filaments, mean esterase activity of self-compatible genotypes increased continuously over the three stages, with the exception of genotype Degumille27. As well, mean esterase activity of filaments from the self-incompatible genotypes Bona12 and Degumille8

increased continuously over the three stages, unlike Bona7 and Degumille9, which showed a different development. The filaments of genotype Degumille9 showed a strong positive reaction (*M = 3,75*) at stage FD1, which subsequently intensified at stage FD2 so that a very strong positive reaction (*M = 5,0*) was observed. Eventually, it decreased at stage FD3 and only a medium positive reaction (*M = 2,8*) was observed. On the other hand, the filaments of genotype Bona7 showed a medium positive reaction (*M = 2,71*) at stage FD1. In the further course of floral development, esterase activity decreased continuously and showed only a weak positive reaction at stage FD2 (*M = 2,33*) and stage FD3 (*M = 2,0*).

**Table 6.5:** Esterase activity of the individual genotypes in different stages of floral development (*ES0*)

| **Papillae** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SC | | | | SI | | | |
| Stage | Bona15 | Bona5 | Deg 27 | Deg 97 | Bona12 | Bona7 | Deg 8 | Deg 9 |
| FD1 | 2.750 (6) | 1.750 (6) | 0.929 (7) | 2.625 (4) | 1.071 (7) | 3.429 (7) | 2.187 (8) | 3.100 (5) |
| FD2 | 3.083 (6) | 1.000 (3) | 2.100 (5) | 1.250 (4) | 3.333 (6) | 1.333 (3) | 2.500 (3) | 1.000 (4) |
| FD3 | 4.500 (5) | 2.125 (4) | 2.750 (4) | 2.375 (4) | 3.500 (4) | 1.857 (7) | 3.167 (3) | 4.900 (5) |
| **Pseudo-Papillae** | | | | | | | | |
| | SC | | | | SI | | | |
| Stage | Bona15 | Bona5 | Deg 27 | Deg 97 | Bona12 | Bona7 | Deg 8 | Deg 9 |
| FD1 | 2.917 (6) | 2.250 (6) | 0.857 (7) | 3.750 (4) | 1.071 (7) | 3.214 (7) | 2.562 (8) | 3.800 (5) |
| FD2 | 1.417 (6) | 0.833 (3) | 2.100 (5) | 0.625 (4) | 0.500 (6) | 1.333 (3) | 2.167 (3) | 0.875 (4) |
| FD3 | 3.500 (5) | 1.625 (4) | 3.250 (4) | 1.625 (4) | 1.875 (4) | 1.786 (7) | 1.333 (3) | 2.800 (5) |
| **Filament** | | | | | | | | |
| | SC | | | | SI | | | |
| Stage | Bona15 | Bona5 | Deg 27 | Deg 97 | Bona12 | Bona7 | Deg 8 | Deg 9 |
| FD1 | 0.667 (6) | 0.333 (6) | 0.857 (7) | 2.250 (4) | 0.429 (7) | 2.714 (7) | 1.375 (8) | 3.750 (4) |
| FD2 | 2.833 (6) | 1.333 (3) | 4.600 (5) | 2.500 (4) | 1.667 (6) | 2.333 (3) | 1.667 (3) | 5.000 (4) |
| FD3 | 3.200 (5) | 1.500 (4) | 3.750 (4) | 4.000 (4) | 2.400 (5) | 2.000 (7) | 2.667 (3) | 2.800 (5) |

Mean values for esterase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) from self-compatible (SC) and self-incompatible (SI) genotypes. The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

By the results of variance analysis (see Table B.1), it was indicated that the factor "genotype and floral development stage" had a statistically highly significant effect on the esterase activity of the papillae and pseudo-papillae ($p < 0,001$), but there was no significant effect on the esterase activity of the filaments ($p > 0,05$). Tukey's HSD test confirmed the results of the variance analysis, however, instead of statistically highly significant differences, only statistically significant differences were found for the MEA of papillae. Concerning the papillae (see Table C.7–C.9), only the differences between the MEA of genotypes Degumille9 and Bona7 at stage FD3 were found to be statistically significant (*diff = 3,04; p = 0,048*).

With regard to the pseudo-papillae (see Table C.10–C.12), there were several statistically significant differences resulting from the multiple comparisons of means, all of which only appeared at stage FD1. Namely, the differences between the MEA of genotypes Degumille9 and Bona12 (*diff = 2,73; p = 0,024*) and between genotypes Degumille27 and Bona7 (*diff = 2,36; p = 0,047*) as well as the differences between the MEA of genotypes Degumille27 and Degumille97 (*diff = 2,89; p = 0,027*) were found to be statistically significant, whereas the differences between the MEA of genotypes Degumille9 and Degumille27 were identified as statistically highly significant (*diff = 2,94; p = 0,0085*).

As for the filaments (see Table C.13–C.15), there were no significant differences resulting from the multiple comparisons of means within the same stages. The effect of the "genotype and

floral development stage" factor on the esterase activity of the papillae, pseudo-papillae and filaments is shown graphically by Figure 6.3.

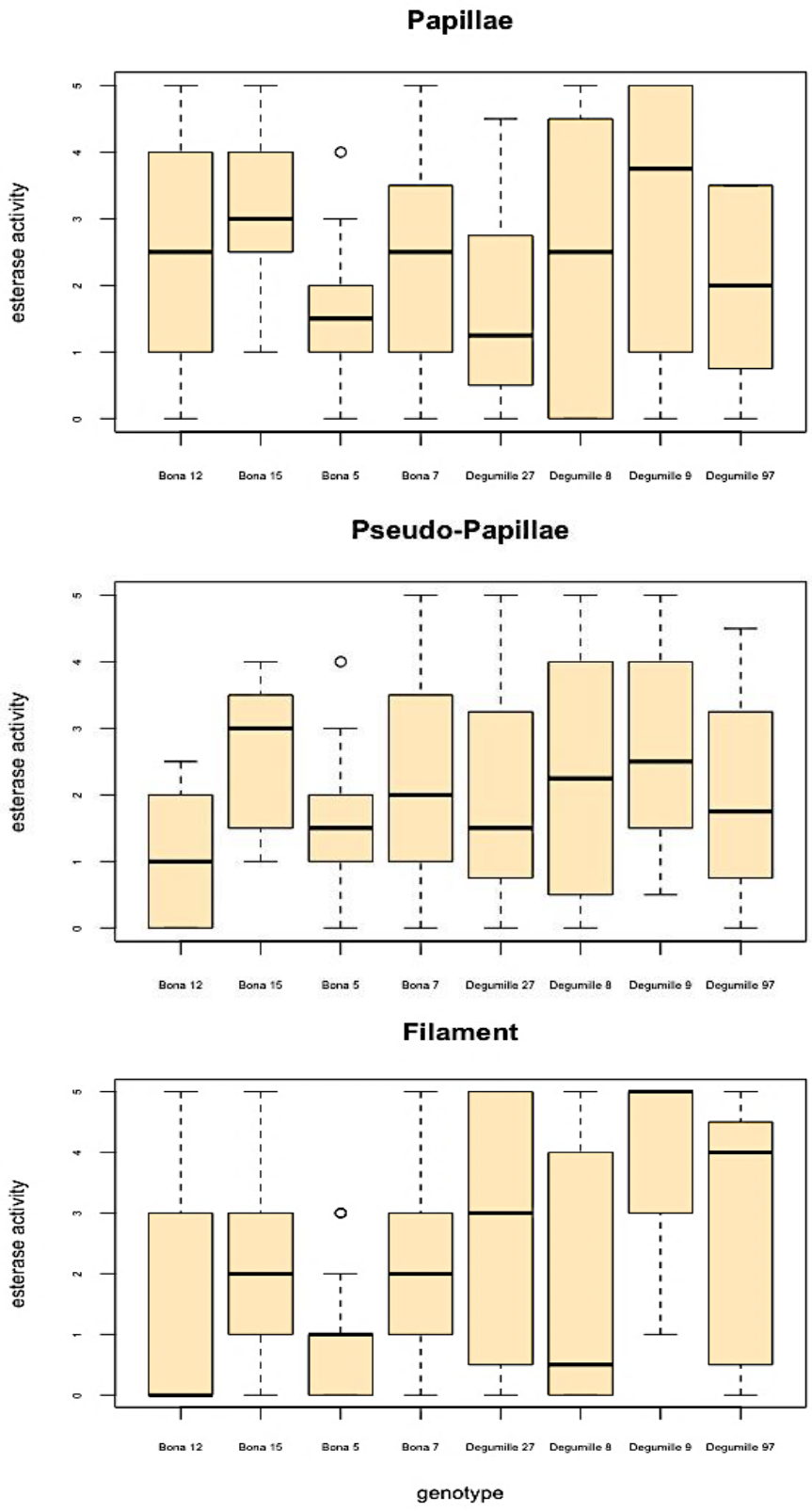


**Figure 6.3:** Genotypic variability (*ES0*). Esterase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) grouped into single genotypes. Genotypes from left to right: Bona12, Bona15, Bona5, Bona7, Degumille27, Degumille8, Degumille9, Degumille97.

## 6.2. Results of test series *ES1*

First, the data from the evaluation of the test series *ES1* were subjected to an analysis of variance (see Appendix B.2, Table B.2). Subsequently, post-hoc comparisons were conducted in order to determine significant differences in the mean esterase activity with regard to the individual factors (see Appendix C.2, Tables C.16–C.30).

### 6.2.1. Effect of the "floral development stage" factor

The values for mean esterase activity (MEA), as can be seen in Table 6.6, reflect a rather weak positive to medium positive reaction of the papillae, pseudo-papillae and filaments during the three stages of floral development. The results of variance analysis (see Table B.2) suggest that the floral development stage had no significant effect on the esterase activity of the papillae but had a statistically highly significant effect on the esterase activity of the pseudo-papillae and filaments. Tukey's HSD test (see Table C.16) confirmed these results.

**Table 6.6:** Esterase activity in different stages of floral development (*ES1*)

| Stage | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| FD1 | 2.668 (47) | 3.057 (47) | 1.244 (47) |
| FD2 | 2.215 (56) | 1.561 (57) | 2.254 (57) |
| FD3 | 2.068 (52) | 1.229 (54) | 3.143 (56) |

Mean values for esterase activity of papillae, pseudo-papillae and filaments in the three stages of floral development (FD1, FD2, FD3). The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

While the MEA of the papillae in stage FD1 ($M = 2{,}67$) was found to be slightly higher than at stage FD2 ($M = 2{,}22$), the differences were not significant ($diff = 0{,}39;\ p > 0{,}05$). Eventually, the MEA of papillae was found to be lowest at stage FD3 ($M = 2{,}07$), whereas both the differences to stage FD2 ($diff = 0{,}25;\ p > 0{,}05$) and the differences to stage FD1 ($diff = 0{,}64;\ p > 0{,}05$) were also found to be statistically not significant.

With regard to the pseudo-papillae, mean esterase activity was again highest at stage FD1 ($M = 3.06$), followed by a decrease at stage FD2 ($M = 1.56$), while the differences between the two stages were identified as statistically highly significant ($diff = 1{,}46;\ p < 0{,}001$). Finally, MEA at stage FD3 ($M = 1.23$) further decreased, but the differences to stage FD2 were not significant ($diff = 0{,}40;\ p > 0{,}05$). On the other hand, the differences between the MEA at stages FD3 and FD1 were found to be statistically highly significant ($diff = 1{,}86;\ p < 0{,}001$).

Unlike the papillae and pseudo-papillae, the MEA of filaments was lowest at stage FD1 ($M = 1.24$), followed by a strong increase at stage FD2 ($M = 2.25$), which caused significant differences between the MEA of the two stages ($diff = 1{,}12;\ p = 0{,}012$). The MEA of filaments peaked at stage FD3 ($M = 3.14$), while the differences to stage FD2 were not significant ($diff = 0{,}81;\ p > 0{,}05$). Then again, the differences between the MEA at stages FD3 and FD1 were found to be statistically highly significant ($diff = 1{,}93;\ p < 0{,}001$).

Additionally, the effect of the "floral development stage" factor on the esterase activity of papillae, pseudo-papillae and filaments is graphically illustrated using Figure 6.4.

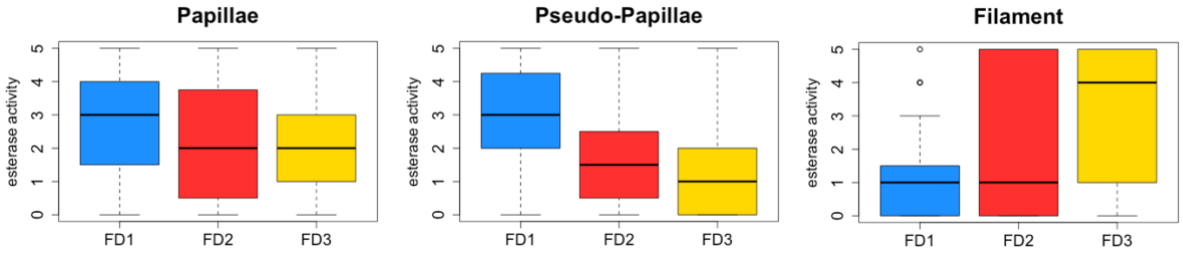


**Figure 6.4:** Variability in esterase activity during floral development (*ES1*). Esterase activity of papillae (left), pseudo-papillae (middle) and filaments (right) in different floral development stages (FD1, FD2, FD3).

### 6.2.2. Effect of the "compatibility" factor

In general, the mean esterase activity (MEA) of self-compatible genotypes was found to be slightly higher compared to that of self-incompatible genotypes, what applies not only to the papillae but also to the pseudo-papillae and filaments (see Table 6.7). The results of variance analysis (see Table B.2) suggest that the "compatibility" factor had a significant influence on the esterase activity of the papillae and pseudo-papillae, but not on the esterase activity of the filaments. However, this was not confirmed by Tukey's HSD test (see Table C.17). Instead, only the papillae showed statistically significant differences when comparing the MEA between SC and SI genotypes (*diff = 0,58; p = 0,019*), unlike the pseudo-papillae (*diff = 0,34; p > 0,05*) and the filaments (*diff = 0,12; p > 0,05*) for which no significant differences were found.

**Table 6.7:** Esterase activity of self-compatible and self-incompatible genotypes (*ES1*)

| Compatibility | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| SC | 2.597 (77) | 2.063 (80) | 2.329 (82) |
| SI | 2.013 (78) | 1.718 (78) | 2.205 (78) |

Mean values for esterase activity of papillae, pseudo-papillae and filaments of self-compatible (SC) and self-incompatible (SI) genotypes. The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

Furthermore, the effect of the "compatibility" factor on the esterase activity of the papillae, pseudo-papillae and filaments is graphically illustrated by Figure 6.5.

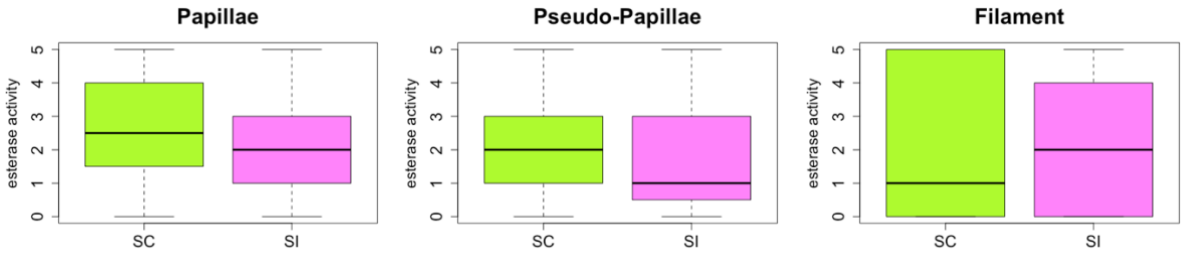


**Figure 6.5:** Variability in esterase activity of self-compatible and self-incompatible genotypes (*ES1*). Esterase activity of papillae (left), pseudo-papillae (middle) and filaments (right) grouped into self-compatible (SC) and self-incompatible (SI) genotypes.

### 6.2.3. Effect of the "compatibility and floral development stage" factor

The results of variance analysis (see Table B.2) indicate a statistically highly significant effect of the "compatibility and stage" factor on the esterase activity of the papillae and pseudo-papillae, but not on the esterase activity of filaments. Tukey's HSD test (see Table C.18) confirmed the results of the variance analysis. When comparing the SC and SI genotypes, there were statistically highly significant differences in the MEA of papillae at stage FD2 (*diff = 0,9;*

*p < 0,001*), as well there were statistically significant differences between the MEA of pseudo-papillae at stage FD1 (*diff = 1,04; p = 0,047*) and statistically highly significant differences at stage FD2 (*diff = -1,38; p < 0,001*). Regarding the filaments, no significant differences were found.

**Table 6.8:** Esterase activity of SC and SI genotypes in different stages of floral development (*ES1*)

| **Papillae** | | |
|---|---|---|
| Stage | SC | SI |
| FD1 | 2.171 (22) | 3.074 (25) |
| FD2 | 3.163 (31) | 1.114 (25) |
| FD3 | 2.258 (24) | 1.868 (28) |
| **Pseudo-Papillae** | | |
| Stage | SC | SI |
| FD1 | 2.49 (22) | 3.53 (25) |
| FD2 | 2.18 (32) | 0.81 (25) |
| FD3 | 1.56 (26) | 0.91 (28) |
| **Filament** | | |
| Stage | SC | SI |
| FD1 | 1.56 (22) | 0.95 (25) |
| FD2 | 2.03 (32) | 2.55 (25) |
| FD3 | 3.27 (28) | 3.01 (28) |

Mean values for the papillae, pseudo-papillae and filaments of self-compatible (SC) and self-incompatible (SI) genotypes at different floral development stages (FD1, FD2, FD3). The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

Table 6.8 displays mean esterase activity (MEA) at the three stages of floral development with distinction between self-compatible (SC) and self-incompatible (SI) genotypes. Regarding the papillae, the MEA of SC genotypes was lowest at stage FD1 (*M = 2,17*), peaked at stage FD2 (*M = 3,16*) and then decreased again in stage FD3 (*M = 2,26*). In contrast, for the self-incompatible genotypes, the MEA of papillae was highest at stage FD1 (*M = 3,07*), strongly decreased in stage FD2 (*M = 1,11*) and then increased again slightly in stage FD3 (*M = 1,87*).

With regard to the pseudo-papillae, the MEA of SC genotypes was highest at stage FD1 (*M = 2,49*), decreased in stage FD2 (*M = 2,18*) and dropped to the lowest level at stage FD3 (*M = 1,56*). For the SI genotypes, the highest MEA was also present at stage FD1 (*M = 3,53*), while it strongly decreased in stage FD2 (*M = 0,81*) and then remained at a low level in stage FD3 (*M = 0,91*). Considering the filaments, the MEA increased continuously over the three stages, both in the self-compatible and self-incompatible genotypes.

### 6.2.4. Effect of the "genotype" factor

As can be seen in Table 6.9, of all SC genotypes, Degumille27 showed the highest MEA of the papillae (*M = 3,09*) and pseudo-papillae (*M = 2,91*), whereas, genotype Bona22 had the highest MEA of the filaments (*M = 3,7*). On the other hand, genotype Bona5 showed the lowest MEA of the papillae (*M = 2,21*), pseudo-papillae (*M = 1,63*) and filaments (*M = 1,08*).

With regard to the SI genotypes, Bona7 had the highest MEA of the papillae (*M = 2,53*) and pseudo-papillae (*M = 2.44*). Genotype Degumille9 showed the highest MEA of the filaments (*M = 2,9*), which was only slightly higher compared to that of genotype Bona7 (*M = 2,83*). Bona12 was found to be the genotype with the lowest MEA of the papillae (*M = 1,68*) and filaments (*M = 1,23*), while genotype Degumille44 had the lowest MEA of the pseudo-papillae (*M = 1,42*).

**Table 6.9:** Esterase activity of the individual genotypes (*ES1*)

| Genotype | Compatibility | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|---|
| Bona19 | SC | 2.867 (15) | 2.000 (16) | 2.444 (18) |
| Bona22 | SC | 2.435 (23) | 1.935 (23) | 3.696 (23) |
| Bona5 | SC | 2.205 (22) | 1.625 (24) | 1.083 (24) |
| Degumille27 | SC | 3.088 (17) | 2.912 (17) | 2.118 (17) |
| Bona12 | SI | 1.682 (22) | 1.591 (22) | 1.227 (22) |
| Bona7 | SI | 2.528 (18) | 2.444 (18) | 2.833 (18) |
| Degumille44 | SI | 1.737 (19) | 1.421 (19) | 2.053 (19) |
| Degumille9 | SI | 2.184 (19) | 1.474 (19) | 2.895 (19) |

Mean values for esterase activity of papillae, pseudo-papillae and filaments of all self-compatible (SC) and self-incompatible (SI) genotypes. The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

The results of variance analysis (see Table B.2) indicates that there was no significant effect of the "genotype" factor on the MEA of papillae, while there was a highly significant effect on the MEA of pseudo-papillae and filaments. However, Tukey's HSD test shows different results. Regarding the MEA of papillae (see Table C.19), significant differences occurred when comparing the SC genotype Degumille27 to the SI genotypes Degumille44 (*diff = 1,34; p = 0,039*) and Bona12 (*diff = 1,4; p = 0,019*).

As for the MEA of pseudo-papillae (see Table C.20), statistically highly significant differences occurred, again when comparing the SC genotype Degumille27 to the SI genotypes Degumille44 (*diff = 1,49; p = 0,0017*) and Bona12 (*diff = 1,33; p = 0,0056*). In addition, statistically highly significant differences were found when comparing the SC genotype Degumille27 to SI genotype Degumille9 (*diff = 1,44; p = 0,0029*) and SC genotype Bona5 (*diff = 1,25; p = 0,0095*). With regard to the MEA of filaments (see Table C.21), statistically significant differences were identified when comparing the SC genotype Bona5 to the SI genotypes Bona7 (*diff = -1,83; p = 0,024*) and Degumille9 (*diff = -1,84; p = 0,019*). Furthermore, statistically highly significant differences were found when comparing the MEA of SC genotype Bona 22 to that of SI genotype Bona12 (*diff = 2,37; p = 0,00037*) and SC genotype Bona5 (*diff = 2,55; p = 0,000056*).

### 6.2.5. Effect of the "genotype and floral development stage" factor

As can be seen in Table 6.10, all genotypes with the exception of Bona5 and Degumille27 showed the highest MEA of the papillae in stage FD1, while it was comparatively lower in stage FD3. It is apparent that the papillae of SC genotypes in stage FD2 showed a relatively high esterase activity and those of SI genotypes were rather reserved. The same pattern applies to the pseudo-papillae. First there was highest activity in stage FD1, which was comparatively lower in stage FD3. The genotype Degumille27 is once again an exception. In general, it can be said that at stage FD2 the esterase activity of pseudo-papillae was again more present in the self-compatible genotypes than in the self-incompatible ones. With respect to the filaments, a reverse pattern appears to apply, since the MEA of each genotype was lower in stage FD1 than in stage FD3.

**Table 6.10:** Esterase activity of the individual genotypes in different stages of floral development (*ES1*)

| **Papillae** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SC | | | | SI | | | |
| Stage | Bona19 | Bona22 | Bona5 | Deg 27 | Bona12 | Bona7 | Deg 44 | Deg 9 |
| FD1 | 3.100 (5) | 3.600 (5) | 1.000 (7) | 1.500 (5) | 2.714 (7) | 3.583 (6) | 2.583 (6) | 3.500 (6) |
| FD2 | 3.071 (7) | 1.90 (10) | 4.071 (7) | 4.286 (7) | 1.286 (7) | 1.333 (6) | 0.500 (6) | 1.333 (6) |
| FD3 | 2.000 (3) | 2.375 (8) | 1.625 (8) | 3.000 (5) | 1.125 (8) | 2.667 (6) | 2.071 (7) | 1.786 (7) |
| **Pseudo-Papillae** | | | | | | | | |
| | SC | | | | SI | | | |
| Stage | Bona19 | Bona22 | Bona5 | Deg 27 | Bona12 | Bona7 | Deg 44 | Deg 9 |
| FD1 | 2.800 (5) | 2.800 (5) | 2.429 (7) | 2.000 (5) | 3.500 (7) | 4.250 (6) | 3.250 (6) | 3.167 (6) |
| FD2 | 2.429 (7) | 1.40 (10) | 2.188 (8) | 3.143 (7) | 0.643 (7) | 1.500 (6) | 0.083 (6) | 1.083 (6) |
| FD3 | 0.250 (4) | 2.062 (8) | 0.500 (9) | 3.500 (5) | 0.750 (8) | 1.583 (6) | 1.000 (7) | 0.357 (7) |
| **Filament** | | | | | | | | |
| | SC | | | | SI | | | |
| Stage | Bona19 | Bona22 | Bona5 | Deg 27 | Bona12 | Bona7 | Deg 44 | Deg 9 |
| FD1 | 2.800 (5) | 2.800 (5) | 0.286 (7) | 0.400 (5) | 0.571 (7) | 0.833 (6) | 0.667 (6) | 1.833 (6) |
| FD2 | 1.429 (7) | 3.60 (10) | 1.500 (8) | 1.429 (7) | 1.000 (7) | 4.167 (6) | 1.833 (6) | 3.500 (6) |
| FD3 | 3.333 (6) | 4.375 (8) | 1.333 (9) | 4.800 (5) | 2.000 (8) | 3.500 (6) | 3.429 (7) | 3.286 (7) |

Mean values for esterase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) from self-compatible (SC) and self-incompatible (SI) genotypes. The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

For the SI genotypes Bona12, Bona7 and Degumille9, strong positive as well as very strong positive reactions were observed in stage FD1, as indicated by the dark staining in the area of the papillae and pseudo-papillae during microscopic analysis (see Figure 6.6). Accordingly, the MEA of the papillae and pseudo-papillae reached a peak in stage FD1. The same applies to the SI genotype Degumille44 (see Table 6.10). During microscopic analysis of the SC genotype Degumille27 (see Figure 6.7), a very strong positive reaction was observed in the area of the papillae in stage FD2, which indicates high esterase activity. Accordingly, the MEA of the papillae not only peaked at stage FD2 ($M = 4{,}3$) but also it was found to be higher compared to that of all other genotypes (see Table 6.10). As well, the microscopic analysis of the SC genotype Bona5 showed a strong positive reaction in stage FD2 (see Figure 6.8), accordingly the MEA of the papillae reached a peak in stage FD2 ($M = 4.07$).

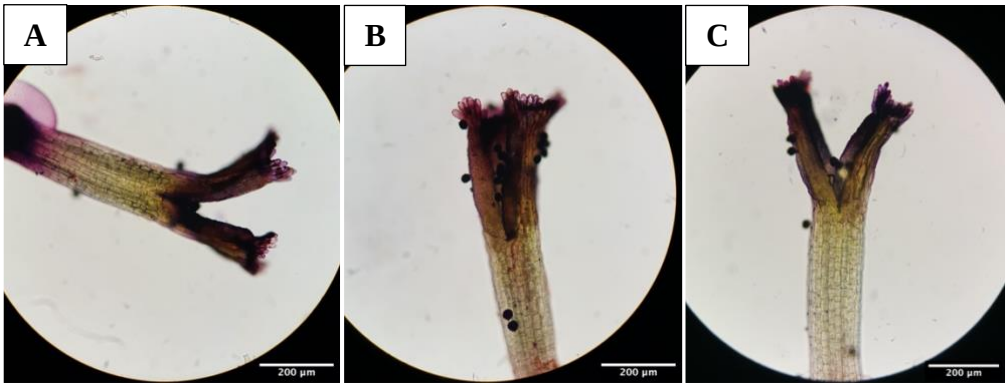


**Figure 6.6:** Stigmatic esterase activity of self-incompatible genotypes in stage FD1. The dark staining in the area of the papillae and pseudo-papillae indicates a strong positive reaction. (A) Genotype Bona12, replication 2. (B) Genotype Degumille9, replication 4. (C) Genotype Bona7, replication 3. Observed under light microscope (Nikon) at 20x magnitude. Bars = 200 µm.

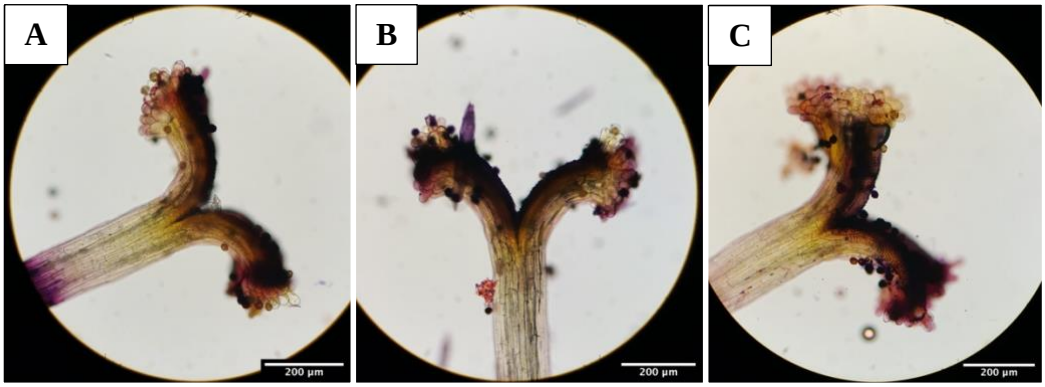


**Figure 6.7:** Stigmatic esterase activity of self-compatible genotype Degumille27 in stage FD2. The dark staining in the area of the papillae indicates a very strong positive reaction. (A) Replication 1. (B) Replication 2. (C) Replication 3. Observed under light microscope (Nikon) at 20x magnitude. Bars = 200 µm.

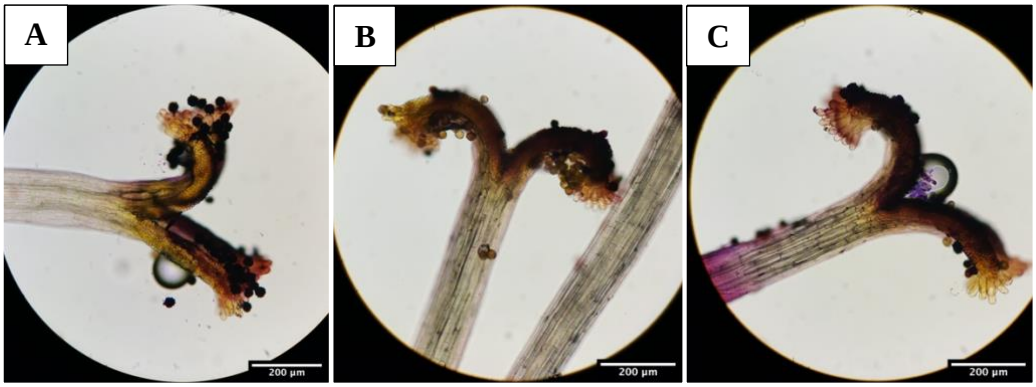


**Figure 6.8:** Stigmatic esterase activity of self-compatible genotype Bona5 in stage FD2. The dark staining in the area of the papillae indicates a strong positive reaction. (A) Replication 3. (B) Replication 4. (C) Replication 5. Observed under light microscope (Nikon) at 20x magnitude. Bars = 200 µm.

The results of variance analysis (see Table B.2) indicate a statistically highly significant effect of the "genotype and floral development stage" factor on the esterase activity of the papillae and pseudo-papillae, but not on the esterase activity of the filaments. This was confirmed by Tukey's HSD test, which revealed statistically significant differences regarding the MEA of papillae (see Table C.22–C.24), all of which only occurred in stage FD2. Namely, when comparing the SC genotype Degumille27 to SI genotype Bona7 (*diff = 2,95; p = 0,011*) and SC genotype Bona22 (*diff = 2,39; p = 0,037*). Further statistically significant differences were found in stage FD2 when comparing the SC genotype Bona5 to SI genotypes Degumille9 (*diff = 2,74; p = 0,03*) and Bona7 (*diff = 2,74; p = 0,03*). Furthermore, statistically highly significant differences were found in stage FD2 when comparing the SC genotype Bona5 with the SI genotype Degumille44 (*diff = 3,57; p = 0,00035*).

With regard to the MEA of pseudo-papillae (see Table C.25–C.27), significant differences occurred in stage FD2 when comparing the SC genotype Bona19 to SI genotype Degumille44 to (*diff = 2,35; p = 0,03*). In addition, highly significant differences were found in stage FD2 when comparing the SC genotype Degumille27 to SI genotype Bona12 (*diff = 2,5; p = 0,007*). Further statistically highly significant differences were identified in stage FD3, all of which were related to SC genotype Degumille27, namely when comparing it to the SC genotypes Bona5 (*diff = 3,00; p = 0,00055*) and Bona19 (*diff = 3,25; p = 0,0039*) as well as in comparison to SI genotype Bona12 (*diff = 2,75; p = 0,0042*). Concerning the MEA of filaments (see Table C.28–C.30), no significant differences were found between individual genotypes within the same stages of floral development.

The effect of the "genotype and floral development stage" factor on the esterase activity of the papillae, pseudo-papillae and filaments is shown graphically by Figure 6.9.

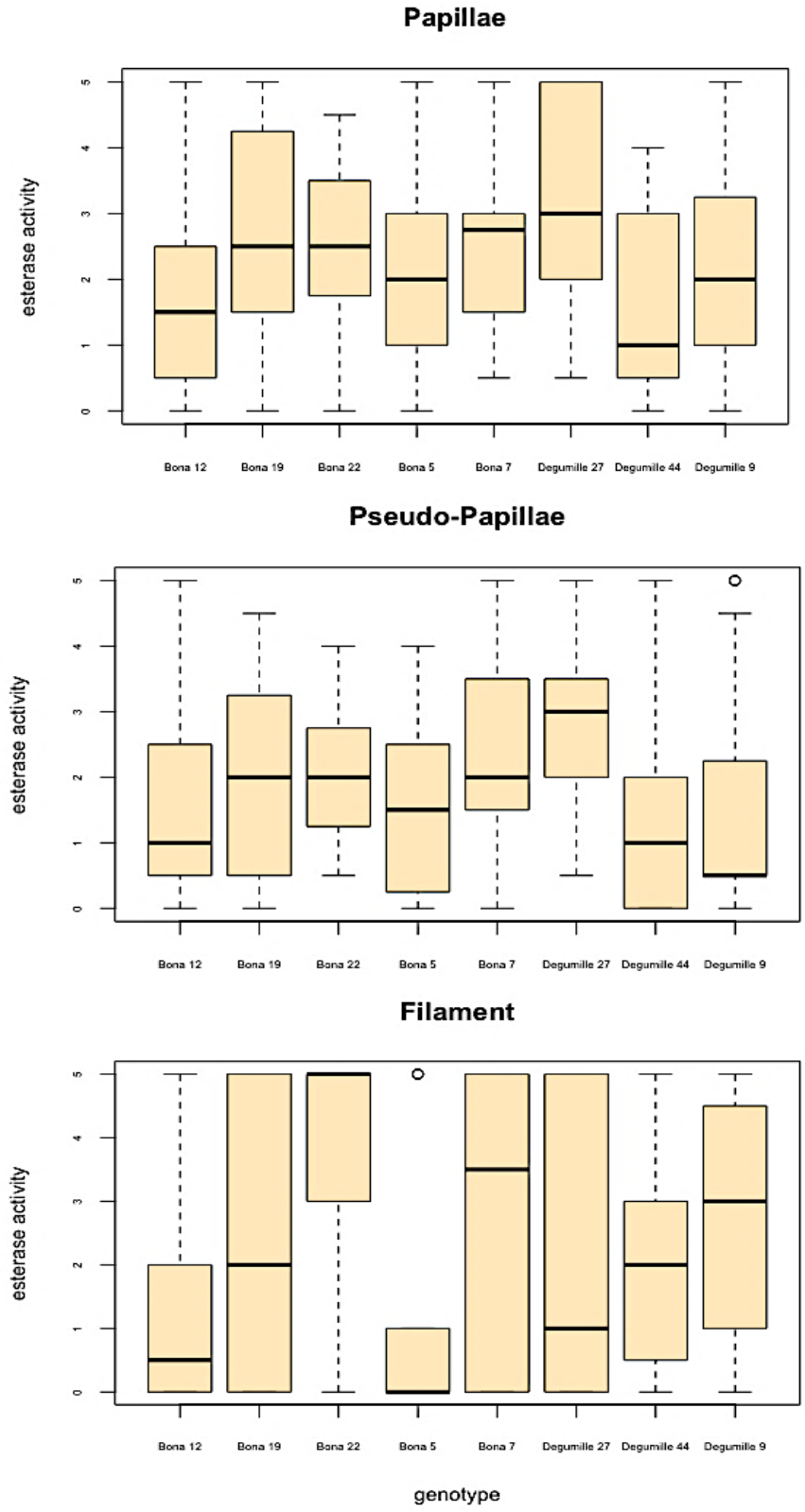


**Figure 6.9:** Genotypic variability (*ES1*). Esterase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) grouped into single genotypes. Genotypes from left to right: Bona12, Bona19, Bona22, Bona5, Bona7, Degumille27, Degumille44, Degumille9.

## 6.3. Results of test series *PE0*

First, the data from the evaluation of the test series *PE0* were subjected to an analysis of variance (see Appendix B.3, Table B.3). Subsequently, post-hoc comparisons were conducted in order to determine significant differences in the mean peroxidase activity with regard to the individual factors (see Appendix C.3, Tables C.31–C.45).

### 6.3.1. Effect of the "floral development stage" factor

In general, the mean peroxidase activity (MPA) of the papillae, pseudo-papillae and filaments was always lowest in stage FD1, then it increased in stage FD2 and peaked at stage FD3 (see Table 6.11). The results of variance analysis (see Table B.3) suggest that the "floral development stage" factor had a statistically highly significant effect on the peroxidase activity of the papillae, pseudo-papillae and filaments. Tukey's HSD test (see Table C.31) confirms that peroxidase activity not only increased continuously over the three stages of floral development, but also differed significantly between the individual stages.

**Table 6.11:** Peroxidase activity in different stages of floral development (*PE0*)

| Stage | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| FD1 | 1.865 (44) | 1.278 (44) | 1.021 (45) |
| FD2 | 2.527 (49) | 1.913 (49) | 1.568 (49) |
| FD3 | 4.002 (43) | 3.093 (43) | 2.587 (43) |

Mean values for peroxidase activity of papillae, pseudo-papillae and filaments from test series PE0, in three stages of floral development (FD1, FD2, FD3). The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

Regarding the MPA of papillae, statistically highly significant differences were found when comparing stage FD1 to stages FD2 (*diff = 0,71; p = 0,0043)* and FD3 (*diff = 2,23*; *p < 0,001)* as well as when comparing stage FD2 to stage FD3 (*diff = 1,52; p < 0,001)*.

As for the MPA of pseudo-papillae, statistically significant differences occurred when comparing stages FD1 and FD2 (*diff = 0,65; p = 0,015*), while statistically highly significant differences were found when comparing stages FD1 and FD3 (*diff = 2,23; p < 0,001*) as well as when comparing stages FD2 and FD3 (*diff = 1,52; p < 0,001*).

With respect to the MPA of filaments, statistically highly significant differences were found when comparing stages FD1 and FD3 (*diff = 1,60; p < 0,001*), as well as when comparing stages FD2 and FD3 (*diff = 1,03; p < 0,0046*). Only when comparing the MPA of filaments between stages FD1 and FD2, no significant differences were found (*diff = 0,57; p > 0,05*).

Additionally, the effect of the "floral development stage" factor on the esterase activity of papillae, pseudo-papillae and filaments is graphically illustrated using Figure 6.10.

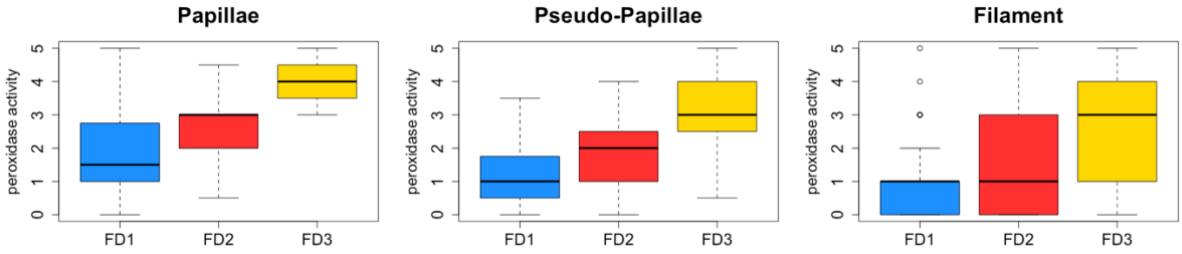


**Figure 6.10:** Variability in peroxidase activity during floral development (*PE0*). Peroxidase activity of papillae (left), pseudo-papillae (middle) and filaments (right) in different floral development stages (FD1, FD2, FD3).

### 6.3.2. Effect of the "compatibility" factor

In general, the mean peroxidase activity (MPA) of self-compatible (SC) and self-incompatible (SI) genotypes indicates a rather weak positive to medium positive reaction (see Table 6.12). Regarding the papillae, the MPA of self-compatible genotypes (*M = 2,59*) was lower compared to that of self-incompatible genotypes (*M = 2,93; diff = 0,34*), whereas the MPA of the pseudo-papillae was found to be almost identical between SC genotypes (*2,06*) and SI genotypes (*M = 2,10; diff = 0,043*). On the other hand, the MPA of filaments was higher in SC genotypes (*M = 1,98*) compared to that of SI genotypes (*M = 1,49; diff = -0,5*).

The results of variance analysis (see Table B.3) suggest that the "compatibility" factor had a significant effect on the peroxidase activity of the papillae and filaments, but not on the peroxidase activity of the pseudo-papillae. However, Tukey's HSD test shows different results (see Table C.32). It was found that there were no statistically significant differences between the mean peroxidase activity of self-compatible and self-incompatible genotypes.

**Table 6.12:** Peroxidase activity of self-compatible and self-incompatible genotypes (*PE0*)

| Compatibility | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| SC | 2.590 (61) | 2.057 (61) | 1.984 (61) |
| SI | 2.933 (75) | 2.100 (75) | 1.487 (76) |

Mean values for peroxidase activity of papillae, pseudo-papillae and filaments of self-compatible (SC) and self-incompatible (SI) genotypes from test series PE0. The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

Furthermore, the effect of the "compatibility" factor on the esterase activity of the papillae, pseudo-papillae and filaments is graphically illustrated by Figure 6.11.

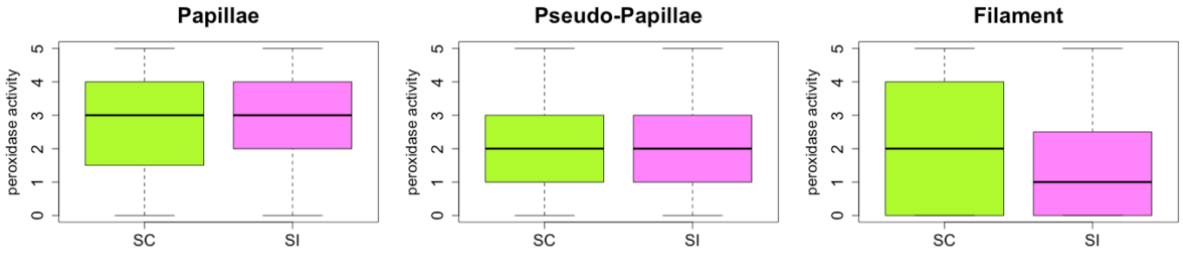


**Figure 6.11:** Variability in peroxidase activity of self-compatible and self-incompatible genotypes (*PE0*). Peroxidase activity of papillae (left), pseudo-papillae (middle) and filaments (right) grouped into self-compatible (SC) and self-incompatible (SI) genotypes.

### 6.3.3. Effect of the "compatibility and floral development stage" factor

As shown in Table 6.13, mean peroxidase activity (MPA) of both self-compatible (SC) and self-incompatible (SI) genotypes was increasing continuously during the three stages of floral development, what applies not only to the papillae but also to the pseudo-papillae and filaments. It can also be seen that the MPA of SC genotypes in stage FD1 was lower compared to that of SI genotypes, whereas it was higher in stages FD2 and FD3. Again, this applies to the papillae, pseudo-papillae and filaments.

The results of variance analysis (see Table B.3) suggest a statistically highly significant effect of the "compatibility and floral development stage" factor on the peroxidase activity of the papillae, pseudo-papillae and filaments. On the other hand, Tukey's HSD test shows different results (see Table C.33). With regard to the MPA of papillae, the differences between SC and SI genotypes in stage FD1 were identified as statistically highly significant (*diff = 1,16; p = 0,0032*). As for the pseudo-papillae, there were no significant differences between the

MPA of SC and SI genotypes within the same stages of floral development. Regarding the MPA of filaments, it was found that the differences between SC and SI genotypes in stage FD3 were statistically highly significant (*diff = 1,73; p = 0,0024*). The remaining comparisons showed no further significant differences.

**Table 6.13:** Peroxidase activity of SC and SI genotypes in different stages of floral development (*PE0*)

| **Papillae** | | |
|---|---|---|
| Stage | SC | SI |
| FD1 | 1.296 (21) | 2.367 (23) |
| FD2 | 2.616 (22) | 2.454 (27) |
| FD3 | 4.069 (18) | 3.972 (25) |
| **Pseudo-Papillae** | | |
| Stage | SC | SI |
| FD1 | 0.933 (21) | 1.592 (23) |
| FD2 | 2.009 (22) | 1.835 (27) |
| FD3 | 3.428 (18) | 2.854 (25) |
| **Filament** | | |
| Stage | SC | SI |
| FD1 | 0.767 (21) | 1.263 (24) |
| FD2 | 1.858 (22) | 1.336 (27) |
| FD3 | 3.557 (18) | 1.865 (25) |

Mean values for peroxidase activities of papillae (top), pseudo-papillae (middle) and filaments (bottom) from self-compatible (SC) and self-incompatible (SI) genotypes in different floral development stages (FD1, FD2, FD3). The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

### 6.3.4. Effect of the "genotype" factor

As can be seen in Table 6.14, of all the self-compatible genotypes, Degumille27 showed the highest MPA of papillae (*M = 2,93*), whereas Bona5 showed the highest MPA of the pseudo-papillae (*M = 2,31*) and filaments (*M = 2,94*). In contrast, Bona15 was found to be the genotype with the lowest MPA of the papillae (*M = 2,14*), pseudo-papillae (*M = 1,77*) and filaments (*M = 1,46*).

As for the self-incompatible genotypes, it was found that Bona12 showed the highest MPA of the papillae (*M = 3,27*) and pseudo-papillae (*M = 3,14*), whereas Degumille9 showed the highest MPA of the filaments (*M = 2,43*). On the other hand, genotype Bona7 had the lowest MPA of the papillae (*M = 2,23*), genotype Degumille9 showed the lowest MPA of the pseudo-papillae (*M = 1,20*), while genotype Degumille8 showed the lowest MPA of the filaments (*M = 0,55*).

**Table 6.14:** Peroxidase activity of the individual genotypes (*PE0*)

| Genotype | Compatibility | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|---|
| Bona15 | SC | 2.136 (22) | 1.773 (22) | 1.455 (22) |
| Bona5 | SC | 2.750 (18) | 2.306 (18) | 2.944 (18) |
| Degumille27 | SC | 2.929 (21) | 2.143 (21) | 1.714 (21) |
| Bona12 | SI | 3.273 (22) | 3.136 (22) | 1.682 (22) |
| Bona7 | SI | 2.231 (13) | 1.885 (13) | 1.077 (13) |
| Degumille8 | SI | 2.900 (20) | 2.000 (20) | 0.550 (20) |
| Degumille9 | SI | 3.050 (20) | 1.200 (20) | 2.429 (21) |

Mean values for peroxidase activity of papillae, pseudo-papillae and filaments of all self-compatible (SC) and self-incompatible (SI) genotypes from test series PE0. The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

The results of variance analysis (see Table B.3) suggest a statistically highly significant effect of the "genotype" factor on the peroxidase activity of the papillae, pseudo-papillae and

filaments. However, Tukey's HSD test shows different results. Regarding the MPA of papillae (see Table C.34), significant differences occurred when comparing SC genotype Bona15 to SI genotype Bona12 (*diff = 0,9; p = 0,011*).

As for the MPA of pseudo-papillae (see Table C.35), significant differences were found, all of which were related to the SI genotypes Bona12 and Degumille9. Interestingly, when comparing the MPA of genotype Bona12 to any other genotype, there were always statistically highly significant differences, namely in the comparison to genotypes Bona15 (*diff = 1,17; p < 0,001*), Bona7 (*diff = 1,1; p = 0,0044*), Degumille27 (*diff = 0,92; p = 0,0072*), Degumille8 (*diff =1,13; p < 0,001*) and Degumille9 (*diff = 1,93; p < 0,001*) with the exception of genotype Bona5, which showed only statistically significant differences (*diff = 0,82; p = 0,036*). All further significant differences were linked to the MPA of genotype Degumille9, namely when comparing it to genotype Degumille8 (*diff = -0,80; p = 0,043*), whereas the comparison to genotypes Degumille27 (*diff = -1,01; p = 0,0029*) and Bona5 (*diff = -1,11; p = 0,0013*) were found to be statistically highly significant.

With respect to the MPA of filaments (see Table C.36), significant differences occurred, namely when comparing the genotype Bona5 to the genotypes Bona12 (*diff = 1,27; p = 0,022*), Bona15 (*diff = 1,33;* p = *0,014*) and Degumille27 (*diff = 1,18; p = 0,049*), while the comparison to the genotypes Bona7 (*diff = 1,74; p = 0,0026*) and Degumille8 (*diff = 2,4; p < 0,001*) even showed statistically highly significant differences. As well, there were significant differences associated with the MPA of genotype Degumille8, which occurred in the comparison to Bona12 (*diff = -1,13; p = 0,04997*) and Degumille27 (*diff = -1,22; p = 0,027*), whereas the comparison to genotype Degumille9 showed statistically highly significant differences (*diff = -1,91; p < 0,001*).

### 6.3.5. Effect of the "genotype and floral development stage" factor

In general, mean peroxidase activity (MPA) of the self-compatible (SC) genotypes increased continuously over the three stages of floral development, which was equally true for the papillae, pseudo-papillae and filaments (see Table 6.15).

**Table 6.15:** Peroxidase activity of the individual genotypes in different stages of floral development (*PE0*)

| **Papillae** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | SC | | | SI | | | |
| Stage | Bona15 | Bona5 | Deg 27 | Bona12 | Bona7 | Deg 8 | Deg 9 |
| FD1 | 1.15 (10) | 0.900 (5) | 1.583 (6) | 2.714 (7) | 2.000 (4) | 1.083 (6) | 3.500 (6) |
| FD2 | 1.583 (6) | 3.357 (7) | 2.889 (9) | 3.071 (7) | 1.917 (6) | 3.071 (7) | 1.500 (7) |
| FD3 | 4.333 (6) | 3.583 (6) | 4.333 (6) | 3.937 (8) | 3.167 (3) | 4.286 (7) | 4.214 (7) |
| **Pseudo-Papillae** | | | | | | | |
| | SC | | | SI | | | |
| Stage | Bona15 | Bona5 | Deg 27 | Bona12 | Bona7 | Deg 8 | Deg 9 |
| FD1 | 1.10 (10) | 0.500 (5) | 0.833 (6) | 2.571 (7) | 2.125 (4) | 0.583 (6) | 1.167 (6) |
| FD2 | 0.917 (6) | 3.000 (7) | 2.056 (9) | 2.857 (7) | 1.167 (6) | 2.500 (7) | 0.571 (7) |
| FD3 | 3.750 (6) | 3.000 (6) | 3.583 (6) | 3.875 (8) | 3.000 (3) | 2.714 (7) | 1.857 (7) |
| **Filament** | | | | | | | |
| | SC | | | SI | | | |
| Stage | Bona15 | Bona5 | Deg 27 | Bona12 | Bona7 | Deg 8 | Deg 9 |
| FD1 | 0.40 (10) | 1.000 (5) | 0.833 (6) | 1.571 (7) | 0.500 (4) | 0.333 (6) | 2.286 (7) |
| FD2 | 0.333 (6) | 3.429 (7) | 1.778 (9) | 0.714 (7) | 2.000 (6) | 0.286 (7) | 2.286 (7) |
| FD3 | 4.333 (6) | 4.000 (6) | 2.500 (6) | 2.625 (8) | 0.000 (3) | 1.000 (7) | 2.714 (7) |

Mean values for peroxidase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) from self-compatible (SC) and self-incompatible (SI) genotypes in different floral development stages (FD1, FD2, FD3). The respective replications are given in brackets. All values result from variance analysis using the statistical program R.

The SC genotype Bona15 was an exception, as the MPA rather stagnated between stages FD1 and FD2 but then increased very sharply between stages FD2 and FD3. The mean peroxidase activity of the self-incompatible (SI) genotypes generally intensified between stages FD1 and FD3, whereas between stages FD1 and FD2 different developments occurred. The filaments of genotype Bona7 were an exception, since the MPA peaked already at stage FD2.

During microscopic analysis of the SI genotype Degumille9 (see Figure 6.12) it was observed that the stigma branches in stage FD1 were partly not yet well developed, but already showed a clearly recognizable staining, which was reflected in a comparatively high MPA of the papillae cells (*M = 3,50*). However, it decreased to a relatively low level at stage FD2 (*M = 1,50*), followed by a dramatic increase in stage FD3 (*M = 4,21*). A rather weak positive to medium positive reaction was detected in the pseudo-papillae cells and the filaments.


**Figure 6.12:** Stigmatic peroxidase activity of self-incompatible genotype *Degumille9*. Peroxidase activity is indicated by the orange-red color, which is lighter or darker depending on the intensity of the reaction. (A-C) Stigmas in floral development stage FD1. (D-F) Stigmas in floral development stage FD2. (G-I) Stigmas in floral development stage FD3. Observed under light microscope (Nikon) at 20x magnitude. Bars = 200 µm.

On the other hand, during microscopic analysis of the SC genotype Bona5 (see Figure 6.13), a rather low positive reaction was observed in the area of the papillae in stage FD1 (*M = 0,90*), which then intensified and was found to be a medium positive to strong positive reaction in stages FD2 (*M = 3,36*) and FD3 (*M = 3,58*). The same applies to the pseudo-papillae and filaments.

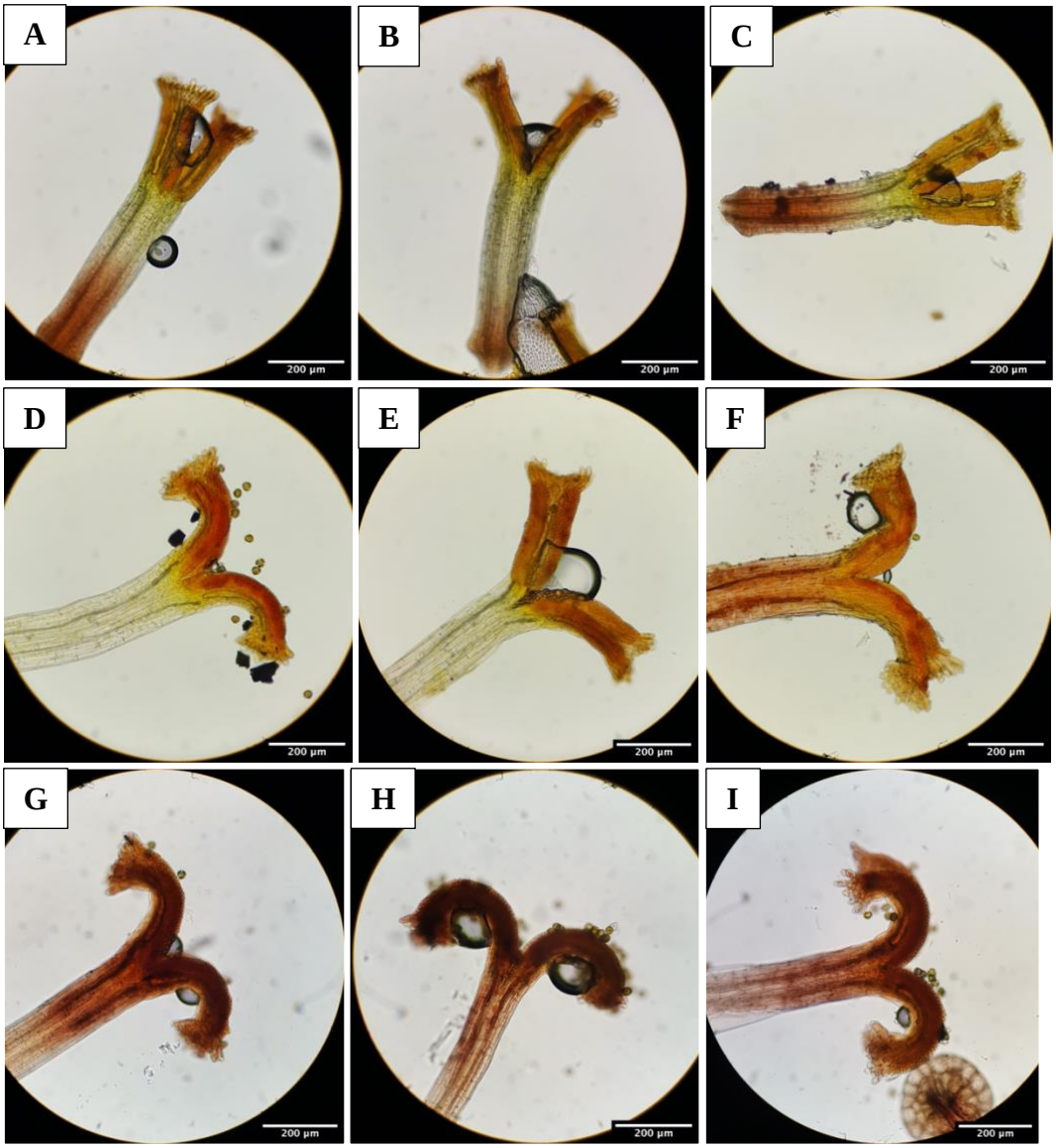


**Figure 6.13:** Stigmatic peroxidase activity of self-compatible genotype Bona5. Peroxidase activity is indicated by the orange-red color, which is lighter or darker depending on the intensity of the reaction. (A-C) Stigmas in floral development stage FD1. (D-F) Stigmas in floral development stage FD2. (G-I) Stigmas in floral development stage FD3. Observed under light microscope (Nikon) at 20x magnitude. Bars = 200 µm.

As indicated by variance analysis (see Table B.3), there was a statistically highly significant effect of the "genotype and floral development stage" factor on the peroxidase activity of the papillae, pseudo-papillae and filaments. The results of variance analysis were confirmed by Tukey's HSD test. Concerning the MPA of papillae in stage FD1 (see Table C.37), significant differences occurred when comparing genotype Bona12 to genotype Bona15 (*diff = 1,56; p = 0,041*), as well as when comparing genotype Degumille9 to genotype Degumille27 (*diff = 1,92; p = 0,024*). In addition, statistically highly significant differences were found when comparing genotype Degumille9 to the genotypes Bona15 (*diff = 2,35; p < 0,001*),

Bona5 (*diff = 2,6; p < 0,001*) and Degumille8 (*diff = 2,42; p < 0,001*). Significant differences also occurred in stage FD2 (see Table C.38), namely in the comparison of genotype Bona5 to the genotypes Degumille9 (*diff = 1,86; p = 0,013*) and Bona15 (*diff = 1,77; p = 0,039)*, whereas no significant differences were found for the MPA of papillae in stage FD3 (see Table C.39).

With regard to the MPA of pseudo-papillae in stage FD1 (see Table C.40), statistically significant differences occurred when comparing the genotype Bona12 to genotype Degumille27 (*diff = 1,74; p = 0,035*), whereas statistically highly significant differences were found when comparing it to genotypes Bona5 (*diff = 2,07; p = 0,0065*) and Degumille8 (*diff = 1,99; p = 0,0055*). In stage FD2 (see Table C.41), significant differences were identified when comparing the genotype Bona12 to genotype Bona7 (*diff = 1,69; p = 0,048*), while statistically highly significant differences occurred when comparing it to genotypes Bona15 (*diff = 1,94; p = 0,008*) and Degumille9 (*diff = 2,29; p = 0,00019*). Further significant differences were revealed in stage FD2, namely when comparing the genotype Bona5 with the genotype Bona7 (*diff = 1,83; p = 0,018*), whereas the comparison to genotypes Bona15 (*diff = 2,08; p = 0,0026*) and Degumille9 (*diff = 2,43; p = 0,000048*) showed statistically highly significant differences, as well as the comparison of genotype Degumille8 with the genotype Degumille9 (*diff = 1,93; p = 0,0047*). For stage FD3 (see Table C.42), significant differences were revealed in the comparison of the genotype Degumille9 with genotypes Bona15 (*diff = -1,89; p = 0,011*) and Degumille27 (*diff = -1,73; p = 0,038*), while the comparison with genotype Bona12 was identified as statistically highly significant (*diff = -2,02; p = 0,0012*).

Regarding the MPA of filaments in stage FD1 (see Table C.43), there were no significant differences between the genotypes. On the other hand, statistically highly significant differences were found in stage FD2 (see Table C.44) when comparing the MPA of genotype Bona5 with that of genotypes Bona12 (*diff = 2,71; p = 0,0092*), Bona15 (*diff = 3,1; p = 0,0021*) and Degumille8 (*diff = 3,14; p = 0,00076*). In stage FD3 (see Table C.45), statistically highly significant differences were identified when comparing the MPA of genotype Bona15 with that of genotypes Bona7 (*diff = 4,33; p = 0,00033*) and Degumille8 (*diff = -3,33; p = 0,00053*), as well as when comparing the MPA of genotype Bona5 with that of genotype Bona7 (*diff = 4,00; p = 0,0016*).

The effect of the “genotype and floral development stage” factor on the peroxidase activity of the papillae, pseudo-papillae and filaments is shown graphically by Figure 6.14.

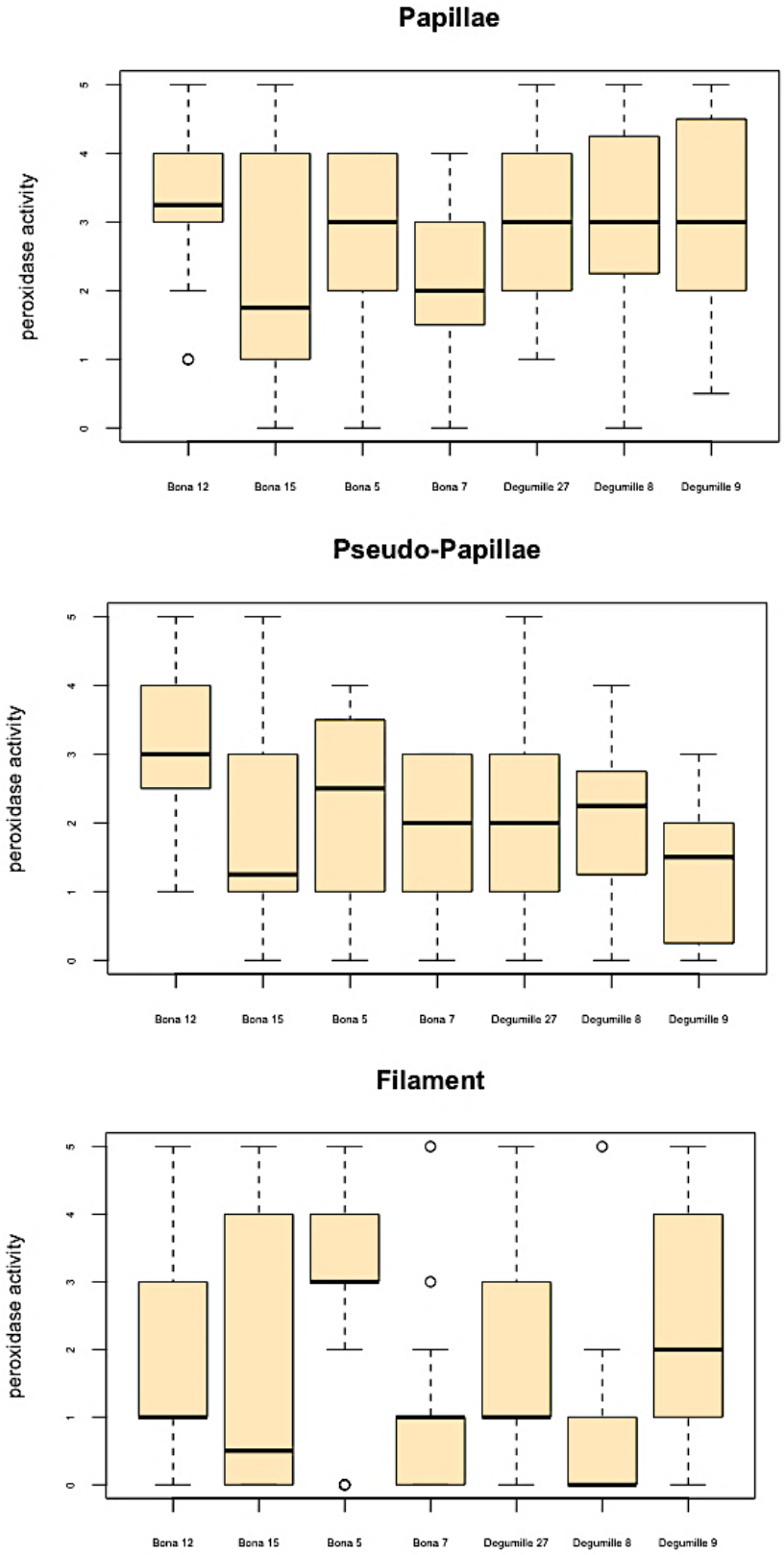


**Figure 6.14:** Genotypic variability (*PE0*). Peroxidase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) grouped into single genotypes. From left to right: Bona12, Bona15, Bona5, Bona7, Degumille27, Degumille8, Degumille9.

## 6.4. Additional results

During microscopic analysis, particularly of the test series *ES0* and *ES1*, the staining reactions could not only be seen in the pistils, but also in the pollen grains. Specifically, it was observed that the pollen reacted in different colors (see Figure 6.15). It can be assumed that the yellow colored pollen grains did not interact with the pistils, since no pollen tube growth could be determined. But it is also possible that the pollen was not yet mature and therefore inactive. On top of that, it cannot be ruled out that this pollen was simply incompatible and therefore no staining reaction could be seen. However, this is rather unlikely, since the pollen grains, when mature, usually themselves have esterases and a staining should therefore be visible. For the red colored pollen grains, however, it can be assumed that they were mature and that an interaction with the pistil was already in progress or at least an associated reaction was imminent and the stronger the reaction, the darker was the staining of the pollen grains.

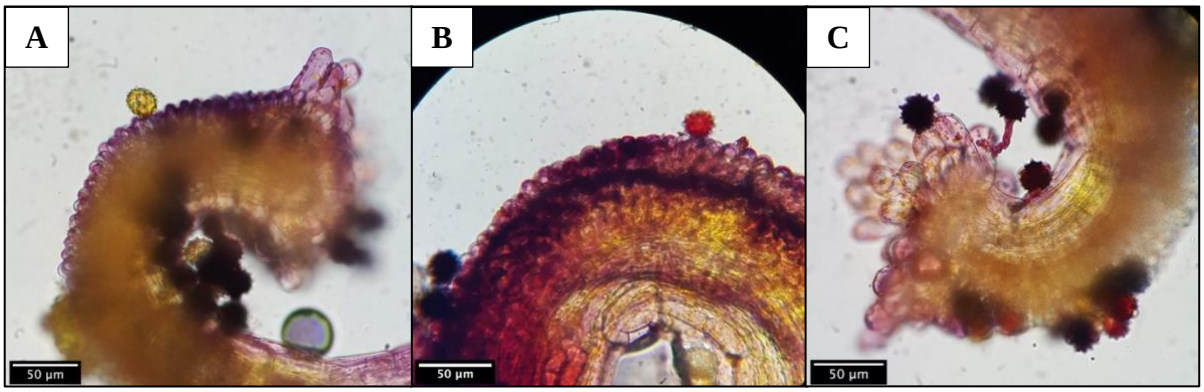


**Figure 6.15:** Different coloring of the pollen grains. A yellow colored pollen grain (A) and a red colored pollen grain (B) adhering to the surface of papillae cells. (C) Very dark red colored pollen grains showing pollen tube formation and penetration into the stigma surface, both into the branch of the stigma and into the pseudo-papillae. Observed under light microscope (Nikon) at 40x magnitude (A, C) and 60x magnitude (B). Bars = 50 µm.

Regardless of the test series, it could also be determined that there was always a gradual separation of the stigmatic branches during the floral development, while the entire apparatus of the pistil increased in size (see Figure 6.16).

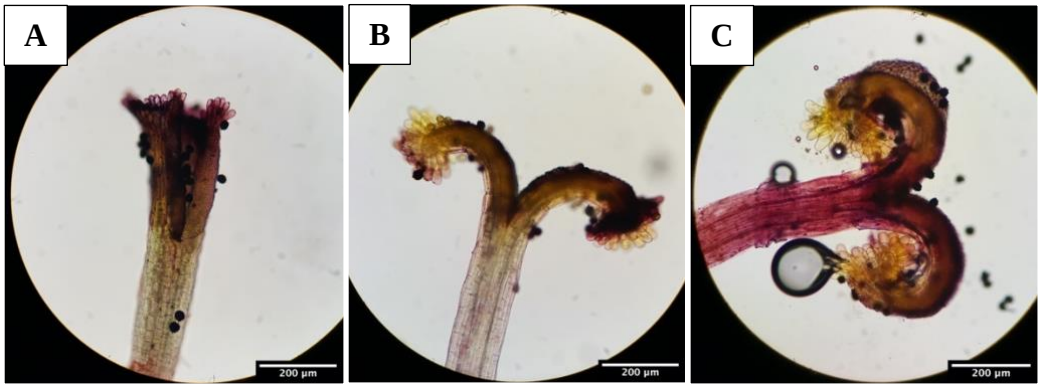


**Figure 6.16:** Gradual separation of the stigmatic branches of genotype Bona7. The stigmatic branches closely appressed at stage FD1 (A), then spread apart at stage FD2 (B) and eventually curled at stage FD3 (C). Observed under light microscope (Nikon) at 20x magnitude. Bars = 200 µm.

# 7. Discussion

As reported by Souza et al. (2016), stigma receptivity occurs only for a short period during the lifetime of a flower, whereas the receptive phase can occur in different stages of floral development. In the present study, the stigmatic activity of esterases and peroxidases was demonstrated for three different stages of floral development, while the intensity of the staining reactions was recorded microscopically and evaluated as a measure of the enzymatic activity. With regard to the intensity of the enzymatic activities, there was a high genotypic variability, which could be determined by significant differences both between the floral development stages and between the individual genotypes. As postulated by McInnis et al. (2006) and Dey et al. (2016), the accumulation of high levels of esterases and peroxidases is a general characteristic of angiosperm stigmas when they are optimally receptive to compatible pollen. Accordingly, no valid statement can be made from the present study regarding the optimal point in time for receptivity to compatible pollen. In general, the stigma receptivity is maximal shortly after the onset of the anthesis, which is also reflected in a high enzymatic activity, but there may be significant differences from species to species (Dey et al., 2016). In the present study, there was generally a constant increase in stigmatic peroxidase activity over the course of floral development. On the other hand, a rather irregular development was observed with regard to the stigmatic esterase activity, which was most likely due to the high genotypic variability.

Furthermore, it could not be determined whether the self-incompatibility reaction was located on the surface of the stigma. For sporophytic systems with a dry stigma, which is believed to be most similar to the self-incompatibility system of German chamomile, it is known that the pollen tube encounters the papillae cells in order to penetrate them, as well as that the following reaction is associated with interactions from cell to cell (De Nettancourt, 2013). In addition, the focusing of the self-incompatibility response is such that a single papilla is able, simultaneously, to accept compatible and reject incompatible pollen (Dickinson, 1995). It would therefore be interesting to know whether individual papillae cells that have been exposed to a pollen-pistil interaction show differences in the activity of peroxidases or esterases when comparing self-compatible and self-incompatible genotypes.

Although the presence of esterases and peroxidases on the stigma surface is considered to be an indication of receptivity, the activity of these enzymes does not confirm the receptivity of the stigma, as the enzymes occur in many stigmas before they can even support pollen germination (Sharma, 2017). In the present study, high enzyme activities could be detected at a very early stage. Some of the stigma branches were not even properly developed. Therefore, it cannot be ruled out that the stigma may not have supported pollen germination at this stage.

If the exact process of the SI mechanism in chamomile should be uncovered, this could be used to increase the profitability of its cultivation. The aim would be to breed a stable, self-incompatible variety which, through selected crossings, could not only lead to a longer-flowering triploid hybrid, but in addition could also have the desired property of being seedless. Obviously, more research is needed to achieve the desired goals. Nonetheless, the present study is intended to provide an insight into the processes of the self-incompatibility mechanism in German chamomile and to take research a step further in this direction.

# 8. Conclusion

Pollen-pistil interactions were provoked by manual self-pollination and the enzymatic activity reactions that took place were investigated. The primary objective was to determine whether the compatibility mechanism has an effect on the activity of the enzymes involved in stigmatic receptivity. For this purpose, histochemical detection of the enzymatic activity at different stages of floral development was carried out. The results indicate that the compatibility mechanism did not have a significant influence on the enzymatic activities. Further research is needed to reveal more details about the compatibility mechanism in German chamomile.

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# Appendix A: Assessment lists

## Appendix A.1: Assessment list of test series *ES0*

**Table A.1:** Assessment list of test series *ES0*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| ES | 0 | SC | Bona 5 | FD1 | 1 | 2 | 2 | 0 |
| ES | 0 | SC | Bona 5 | FD1 | 2 | 4 | 4 | 0 |
| ES | 0 | SC | Bona 5 | FD1 | 3 | 3 | 2 | 0 |
| ES | 0 | SC | Bona 5 | FD1 | 4 | 2 | 1 | 0 |
| ES | 0 | SC | Bona 5 | FD1 | 5 | 1 | 1 | 1 |
| ES | 0 | SC | Bona 5 | FD1 | 6 | 2 | 1 | 1 |
| ES | 0 | SC | Bona 5 | FD2 | 1 | 0 | 0 | 0 |
| ES | 0 | SC | Bona 5 | FD2 | 2 | 1 | 1 | 3 |
| ES | 0 | SC | Bona 5 | FD2 | 3 | 2 | 2 | 1 |
| ES | 0 | SC | Bona 5 | FD3 | 1 | 3 | 3 | 3 |
| ES | 0 | SC | Bona 5 | FD3 | 2 | 2 | 2 | 2 |
| ES | 0 | SC | Bona 5 | FD3 | 3 | 1 | 2 | 0 |
| ES | 0 | SC | Bona 5 | FD3 | 4 | 1 | 2 | 1 |
| ES | 0 | SC | Bona 15 | FD1 | 1 | 3 | 3 | 0 |
| ES | 0 | SC | Bona 15 | FD1 | 2 | 3 | 3 | 0 |
| ES | 0 | SC | Bona 15 | FD1 | 3 | 4 | 4 | 1 |
| ES | 0 | SC | Bona 15 | FD1 | 4 | 4 | 4 | 2 |
| ES | 0 | SC | Bona 15 | FD1 | 5 | 2 | 2 | 0 |
| ES | 0 | SC | Bona 15 | FD1 | 6 | 2 | 1 | 1 |
| ES | 0 | SC | Bona 15 | FD2 | 1 | 2 | 4 | 1 |
| ES | 0 | SC | Bona 15 | FD2 | 2 | 2 | 3 | 0 |
| ES | 0 | SC | Bona 15 | FD2 | 3 | 1 | 3 | 5 |
| ES | 0 | SC | Bona 15 | FD2 | 4 | 1 | 3 | 1 |
| ES | 0 | SC | Bona 15 | FD2 | 5 | 1 | 3 | 5 |
| ES | 0 | SC | Bona 15 | FD2 | 6 | 3 | 4 | 5 |
| ES | 0 | SC | Bona 15 | FD3 | 1 | 3 | 3 | 3 |
| ES | 0 | SC | Bona 15 | FD3 | 2 | 4 | 5 | 3 |
| ES | 0 | SC | Bona 15 | FD3 | 3 | 4 | 5 | 3 |
| ES | 0 | SC | Bona 15 | FD3 | 4 | 4 | 5 | 4 |
| ES | 0 | SC | Bona 15 | FD3 | 5 | 4 | 5 | 3 |
| ES | 0 | SC | Degumille 27 | FD1 | 1 | 1 | 1 | 0 |
| ES | 0 | SC | Degumille 27 | FD1 | 2 | 1 | 1 | 1 |
| ES | 0 | SC | Degumille 27 | FD1 | 3 | 2 | 2 | 2 |
| ES | 0 | SC | Degumille 27 | FD1 | 4 | 2 | 1 | 1 |
| ES | 0 | SC | Degumille 27 | FD1 | 5 | 2 | 2 | 2 |
| ES | 0 | SC | Degumille 27 | FD1 | 6 | 0 | 1 | 0 |
| ES | 0 | SC | Degumille 27 | FD1 | 7 | 0 | 0 | 0 |
| ES | 0 | SC | Degumille 27 | FD2 | 1 | 1 | 2 | 4 |
| ES | 0 | SC | Degumille 27 | FD2 | 2 | 1 | 1 | 4 |
| ES | 0 | SC | Degumille 27 | FD2 | 3 | 5 | 5 | 5 |
| ES | 0 | SC | Degumille 27 | FD2 | 4 | 3 | 3 | 5 |
| ES | 0 | SC | Degumille 27 | FD2 | 5 | 1 | 1 | 5 |
| ES | 0 | SC | Degumille 27 | FD3 | 1 | 4 | 3 | 0 |
| ES | 0 | SC | Degumille 27 | FD3 | 2 | 2 | 0 | 5 |
| ES | 0 | SC | Degumille 27 | FD3 | 3 | 5 | 4 | 5 |
| ES | 0 | SC | Degumille 27 | FD3 | 4 | 4 | 5 | 5 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

**Table A.1 (continued):** Assessment list of test series *ES0*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| ES | 0 | SC | Degumille 97 | FD1 | 1 | 3 | 0 | 1 |
| ES | 0 | SC | Degumille 97 | FD1 | 2 | 5 | 4 | 0 |
| ES | 0 | SC | Degumille 97 | FD1 | 3 | 4 | 4 | 3 |
| ES | 0 | SC | Degumille 97 | FD1 | 4 | 4 | 4 | 5 |
| ES | 0 | SC | Degumille 97 | FD2 | 1 | 2 | 2 | 0 |
| ES | 0 | SC | Degumille 97 | FD2 | 2 | 0 | 2 | 5 |
| ES | 0 | SC | Degumille 97 | FD2 | 3 | 1 | 1 | 0 |
| ES | 0 | SC | Degumille 97 | FD2 | 4 | 1 | 1 | 5 |
| ES | 0 | SC | Degumille 97 | FD3 | 1 | 2 | 4 | 4 |
| ES | 0 | SC | Degumille 97 | FD3 | 2 | 2 | 3 | 4 |
| ES | 0 | SC | Degumille 97 | FD3 | 3 | 1 | 1 | 4 |
| ES | 0 | SC | Degumille 97 | FD3 | 4 | 2 | 2 | 4 |
| ES | 0 | SI | Bona 7 | FD1 | 1 | 4 | 5 | 3 |
| ES | 0 | SI | Bona 7 | FD1 | 2 | 4 | 2 | 0 |
| ES | 0 | SI | Bona 7 | FD1 | 3 | 4 | 4 | 1 |
| ES | 0 | SI | Bona 7 | FD1 | 4 | 1 | 4 | 5 |
| ES | 0 | SI | Bona 7 | FD1 | 5 | 5 | 5 | 5 |
| ES | 0 | SI | Bona 7 | FD1 | 6 | 5 | 5 | 5 |
| ES | 0 | SI | Bona 7 | FD1 | 7 | 1 | 0 | 0 |
| ES | 0 | SI | Bona 7 | FD2 | 1 | 2 | 3 | 3 |
| ES | 0 | SI | Bona 7 | FD2 | 2 | 1 | 1 | 1 |
| ES | 0 | SI | Bona 7 | FD2 | 3 | 2 | 1 | 3 |
| ES | 0 | SI | Bona 7 | FD3 | 1 | 3 | 2 | 1 |
| ES | 0 | SI | Bona 7 | FD3 | 2 | 2 | 3 | 3 |
| ES | 0 | SI | Bona 7 | FD3 | 3 | 2 | 3 | 2 |
| ES | 0 | SI | Bona 7 | FD3 | 4 | 2 | 3 | 1 |
| ES | 0 | SI | Bona 7 | FD3 | 5 | 0 | 0 | 5 |
| ES | 0 | SI | Bona 7 | FD3 | 6 | 3 | 2 | 1 |
| ES | 0 | SI | Bona 7 | FD3 | 7 | 1 | 1 | 1 |
| ES | 0 | SI | Bona 12 | FD1 | 1 | 2 | 2 | 1 |
| ES | 0 | SI | Bona 12 | FD1 | 2 | 0 | 1 | 0 |
| ES | 0 | SI | Bona 12 | FD1 | 3 | 3 | 3 | 0 |
| ES | 0 | SI | Bona 12 | FD1 | 4 | 1 | 0 | 2 |
| ES | 0 | SI | Bona 12 | FD1 | 5 | 1 | 1 | 0 |
| ES | 0 | SI | Bona 12 | FD1 | 6 | 1 | 1 | 0 |
| ES | 0 | SI | Bona 12 | FD1 | 7 | 1 | 1 | 0 |
| ES | 0 | SI | Bona 12 | FD2 | 1 | 0 | 2 | 0 |
| ES | 0 | SI | Bona 12 | FD2 | 2 | 1 | 5 | 0 |
| ES | 0 | SI | Bona 12 | FD2 | 3 | 0 | 2 | 0 |
| ES | 0 | SI | Bona 12 | FD2 | 4 | 0 | 4 | 5 |
| ES | 0 | SI | Bona 12 | FD2 | 5 | 0 | 3 | 0 |
| ES | 0 | SI | Bona 12 | FD2 | 6 | 3 | 5 | 5 |
| ES | 0 | SI | Bona 12 | FD3 | 1 | 2 | 3 | 3 |
| ES | 0 | SI | Bona 12 | FD3 | 2 | 2 | 4 | 1 |
| ES | 0 | SI | Bona 12 | FD3 | 3 | | | 0 |
| ES | 0 | SI | Bona 12 | FD3 | 4 | 2 | 4 | 5 |
| ES | 0 | SI | Bona 12 | FD3 | 5 | 2 | 4 | 3 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

**Table A.1 (continued):** Assessment list of test series *ES0*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| ES | 0 | SI | Degumille 8 | FD1 | 1 | 5 | 5 | 2 |
| ES | 0 | SI | Degumille 8 | FD1 | 2 | 5 | 5 | 3 |
| ES | 0 | SI | Degumille 8 | FD1 | 3 | 3 | 2 | 0 |
| ES | 0 | SI | Degumille 8 | FD1 | 4 | 2 | 0 | 0 |
| ES | 0 | SI | Degumille 8 | FD1 | 5 | 0 | 1 | 1 |
| ES | 0 | SI | Degumille 8 | FD1 | 6 | 5 | 5 | 5 |
| ES | 0 | SI | Degumille 8 | FD1 | 7 | 1 | 0 | 0 |
| ES | 0 | SI | Degumille 8 | FD1 | 8 | 1 | 0 | 0 |
| ES | 0 | SI | Degumille 8 | FD2 | 1 | 3 | 3 | 0 |
| ES | 0 | SI | Degumille 8 | FD2 | 2 | 0 | 0 | 0 |
| ES | 0 | SI | Degumille 8 | FD2 | 3 | 4 | 5 | 5 |
| ES | 0 | SI | Degumille 8 | FD3 | 1 | 1 | 3 | 0 |
| ES | 0 | SI | Degumille 8 | FD3 | 2 | 3 | 4 | 4 |
| ES | 0 | SI | Degumille 8 | FD3 | 3 | 1 | 4 | 4 |
| ES | 0 | SI | Degumille 9 | FD1 | 1 | 2 | 3 | 2 |
| ES | 0 | SI | Degumille 9 | FD1 | 2 | 5 | 0 | |
| ES | 0 | SI | Degumille 9 | FD1 | 3 | 5 | 4 | 3 |
| ES | 0 | SI | Degumille 9 | FD1 | 4 | 3 | 4 | 5 |
| ES | 0 | SI | Degumille 9 | FD1 | 5 | 5 | 5 | 5 |
| ES | 0 | SI | Degumille 9 | FD2 | 1 | 1 | 1 | 5 |
| ES | 0 | SI | Degumille 9 | FD2 | 2 | 2 | 3 | 5 |
| ES | 0 | SI | Degumille 9 | FD2 | 3 | 1 | 0 | 5 |
| ES | 0 | SI | Degumille 9 | FD2 | 4 | 1 | 1 | 5 |
| ES | 0 | SI | Degumille 9 | FD3 | 1 | 3 | 5 | 5 |
| ES | 0 | SI | Degumille 9 | FD3 | 2 | 4 | 5 | 1 |
| ES | 0 | SI | Degumille 9 | FD3 | 3 | 3 | 5 | 1 |
| ES | 0 | SI | Degumille 9 | FD3 | 4 | 3 | 5 | 3 |
| ES | 0 | SI | Degumille 9 | FD3 | 5 | 2 | 5 | 4 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

# Appendix A.2: Assessment list of test series *ES1*

**Table A.2:** Assessment list of test series *ES1*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| ES | 1 | SC | Bona 5 | FD1 | 1 | 2 | 0 | 0 |
| ES | 1 | SC | Bona 5 | FD1 | 2 | 3 | 1 | 0 |
| ES | 1 | SC | Bona 5 | FD1 | 3 | 3 | 1 | 1 |
| ES | 1 | SC | Bona 5 | FD1 | 4 | 2 | 0 | 0 |
| ES | 1 | SC | Bona 5 | FD1 | 5 | 4 | 3 | 0 |
| ES | 1 | SC | Bona 5 | FD1 | 6 | 4 | 2 | 1 |
| ES | 1 | SC | Bona 5 | FD1 | 7 | 1 | 1 | 0 |
| ES | 1 | SC | Bona 5 | FD2 | 1 | 1 |  | 0 |
| ES | 1 | SC | Bona 5 | FD2 | 2 | 2 | 3 | 0 |
| ES | 1 | SC | Bona 5 | FD2 | 3 | 3 | 4 | 0 |
| ES | 1 | SC | Bona 5 | FD2 | 4 | 2 | 3 | 1 |
| ES | 1 | SC | Bona 5 | FD2 | 5 | 2 | 5 | 1 |
| ES | 1 | SC | Bona 5 | FD2 | 6 | 4 | 5 | 0 |
| ES | 1 | SC | Bona 5 | FD2 | 7 | 3 | 5 | 5 |
| ES | 1 | SC | Bona 5 | FD2 | 8 | 3 | 5 | 5 |
| ES | 1 | SC | Bona 5 | FD3 | 1 | 0 | 2 | 1 |
| ES | 1 | SC | Bona 5 | FD3 | 2 | 0 | 2 | 0 |
| ES | 1 | SC | Bona 5 | FD3 | 3 | 0 | 2 | 5 |
| ES | 1 | SC | Bona 5 | FD3 | 4 | 0 | 0 | 0 |
| ES | 1 | SC | Bona 5 | FD3 | 5 | 1 | 2 | 5 |
| ES | 1 | SC | Bona 5 | FD3 | 6 | 2 | 2 | 0 |
| ES | 1 | SC | Bona 5 | FD3 | 7 | 2 | 3 | 0 |
| ES | 1 | SC | Bona 5 | FD3 | 8 | 0 | 1 | 0 |
| ES | 1 | SC | Bona 5 | FD3 | 9 | 0 |  | 1 |
| ES | 1 | SC | Bona 19 | FD1 | 1 | 2 | 2 | 1 |
| ES | 1 | SC | Bona 19 | FD1 | 2 | 3 | 3 | 1 |
| ES | 1 | SC | Bona 19 | FD1 | 3 | 5 | 5 | 3 |
| ES | 1 | SC | Bona 19 | FD1 | 4 | 1 | 2 | 5 |
| ES | 1 | SC | Bona 19 | FD1 | 5 | 5 | 5 | 4 |
| ES | 1 | SC | Bona 19 | FD2 | 1 | 4 | 4 | 0 |
| ES | 1 | SC | Bona 19 | FD2 | 2 | 1 | 2 | 0 |
| ES | 1 | SC | Bona 19 | FD2 | 3 | 1 | 3 | 0 |
| ES | 1 | SC | Bona 19 | FD2 | 4 | 3 | 3 | 0 |
| ES | 1 | SC | Bona 19 | FD2 | 5 | 3 | 2 | 0 |
| ES | 1 | SC | Bona 19 | FD2 | 6 | 4 | 5 | 5 |
| ES | 1 | SC | Bona 19 | FD2 | 7 | 3 | 4 | 5 |
| ES | 1 | SC | Bona 19 | FD3 | 1 | 0 | 1 | 4 |
| ES | 1 | SC | Bona 19 | FD3 | 2 |  |  | 5 |
| ES | 1 | SC | Bona 19 | FD3 | 3 | 1 | 0 | 0 |
| ES | 1 | SC | Bona 19 | FD3 | 4 | 0 | 5 | 1 |
| ES | 1 | SC | Bona 19 | FD3 | 5 | 0 |  | 5 |
| ES | 1 | SC | Bona 19 | FD3 | 6 |  |  | 5 |
| ES | 1 | SC | Bona 22 | FD1 | 1 | 1 | 4 | 0 |
| ES | 1 | SC | Bona 22 | FD1 | 2 | 3 | 3 | 5 |
| ES | 1 | SC | Bona 22 | FD1 | 3 | 4 | 5 | 4 |
| ES | 1 | SC | Bona 22 | FD1 | 4 | 2 | 3 | 4 |
| ES | 1 | SC | Bona 22 | FD1 | 5 | 4 | 4 | 1 |
| ES | 1 | SC | Bona 22 | FD2 | 1 | 1 | 0 | 5 |
| ES | 1 | SC | Bona 22 | FD2 | 2 | 1 | 0 | 0 |
| ES | 1 | SC | Bona 22 | FD2 | 3 | 1 | 2 | 1 |
| ES | 1 | SC | Bona 22 | FD2 | 4 | 2 | 2 | 5 |
| ES | 1 | SC | Bona 22 | FD2 | 5 | 1 | 0 | 0 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

**Table A.2 (continued):** Assessment list of test series *ES1*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| ES | 1 | SC | Bona 22 | FD2 | 6 | 1 | 1 | 5 |
| ES | 1 | SC | Bona 22 | FD2 | 7 | 3 | 4 | 5 |
| ES | 1 | SC | Bona 22 | FD2 | 8 | 2 | 4 | 5 |
| ES | 1 | SC | Bona 22 | FD2 | 9 | 2 | 4 | 5 |
| ES | 1 | SC | Bona 22 | FD2 | 10 | 3 | 4 | 5 |
| ES | 1 | SC | Bona 22 | FD3 | 1 | 3 | 4 | 4 |
| ES | 1 | SC | Bona 22 | FD3 | 2 | 2 | 3 | 2 |
| ES | 1 | SC | Bona 22 | FD3 | 3 | 2 | 1 | 5 |
| ES | 1 | SC | Bona 22 | FD3 | 4 | 2 | 4 | 5 |
| ES | 1 | SC | Bona 22 | FD3 | 5 | 2 | 2 | 5 |
| ES | 1 | SC | Bona 22 | FD3 | 6 | 2 | 2 | 4 |
| ES | 1 | SC | Bona 22 | FD3 | 7 | 2 | 2 | 5 |
| ES | 1 | SC | Bona 22 | FD3 | 8 | 3 | 2 | 5 |
| ES | 1 | SC | Degumille 27 | FD1 | 1 | 1 | 1 | 1 |
| ES | 1 | SC | Degumille 27 | FD1 | 2 | 3 | 1 | 0 |
| ES | 1 | SC | Degumille 27 | FD1 | 3 | 2 | 1 | 0 |
| ES | 1 | SC | Degumille 27 | FD1 | 4 | 2 | 2 | 1 |
| ES | 1 | SC | Degumille 27 | FD1 | 5 | 3 | 3 | 0 |
| ES | 1 | SC | Degumille 27 | FD2 | 1 | 3 | 5 | 0 |
| ES | 1 | SC | Degumille 27 | FD2 | 2 | 3 | 5 | 0 |
| ES | 1 | SC | Degumille 27 | FD2 | 3 | 4 | 5 | 0 |
| ES | 1 | SC | Degumille 27 | FD2 | 4 | 4 | 5 | 1 |
| ES | 1 | SC | Degumille 27 | FD2 | 5 | 1 | 1 | 0 |
| ES | 1 | SC | Degumille 27 | FD2 | 6 | 4 | 5 | 5 |
| ES | 1 | SC | Degumille 27 | FD2 | 7 | 5 | 5 | 4 |
| ES | 1 | SC | Degumille 27 | FD3 | 1 | 4 | 2 | 5 |
| ES | 1 | SC | Degumille 27 | FD3 | 2 | 2 | 3 | 5 |
| ES | 1 | SC | Degumille 27 | FD3 | 3 | 5 | 3 | 5 |
| ES | 1 | SC | Degumille 27 | FD3 | 4 | 5 | 4 | 5 |
| ES | 1 | SC | Degumille 27 | FD3 | 5 | 3 | 3 | 4 |
| ES | 1 | SI | Bona 7 | FD1 | 1 | 5 | 4 | 0 |
| ES | 1 | SI | Bona 7 | FD1 | 2 | 5 | 4 | 0 |
| ES | 1 | SI | Bona 7 | FD1 | 3 | 3 | 3 | 0 |
| ES | 1 | SI | Bona 7 | FD1 | 4 | 5 | 5 | 3 |
| ES | 1 | SI | Bona 7 | FD1 | 5 | 5 | 3 | 2 |
| ES | 1 | SI | Bona 7 | FD1 | 6 | 3 | 3 | 0 |
| ES | 1 | SI | Bona 7 | FD2 | 1 | 2 | 1 | 5 |
| ES | 1 | SI | Bona 7 | FD2 | 2 | 2 | 2 | 5 |
| ES | 1 | SI | Bona 7 | FD2 | 3 | 3 | 1 | 5 |
| ES | 1 | SI | Bona 7 | FD2 | 4 | 1 | 1 | 5 |
| ES | 1 | SI | Bona 7 | FD2 | 5 | 1 | 2 | 0 |
| ES | 1 | SI | Bona 7 | FD2 | 6 | 2 | 3 | 5 |
| ES | 1 | SI | Bona 7 | FD3 | 1 | 4 | 3 | 5 |
| ES | 1 | SI | Bona 7 | FD3 | 2 | 1 | 1 | 3 |
| ES | 1 | SI | Bona 7 | FD3 | 3 | 0 | 3 | 4 |
| ES | 1 | SI | Bona 7 | FD3 | 4 | 3 | 4 | 4 |
| ES | 1 | SI | Bona 7 | FD3 | 5 | 2 | 3 | 4 |
| ES | 1 | SI | Bona 7 | FD3 | 6 | 2 | 3 | 1 |
| ES | 1 | SI | Bona 12 | FD1 | 1 | 4 | 2 | 0 |
| ES | 1 | SI | Bona 12 | FD1 | 2 | 5 | 5 | 1 |
| ES | 1 | SI | Bona 12 | FD1 | 3 | 2 | 2 | 0 |
| ES | 1 | SI | Bona 12 | FD1 | 4 | 3 | 1 | 0 |
| ES | 1 | SI | Bona 12 | FD1 | 5 | 3 | 3 | 1 |
| ES | 1 | SI | Bona 12 | FD1 | 6 | 4 | 3 | 1 |
| ES | 1 | SI | Bona 12 | FD1 | 7 | 5 | 4 | 1 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

**Table A.2 (continued):** Assessment list of test series *ES1*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| ES | 1 | SI | Bona 12 | FD2 | 1 | 0 | 0 | 0 |
| ES | 1 | SI | Bona 12 | FD2 | 2 | 2 | 4 | 0 |
| ES | 1 | SI | Bona 12 | FD2 | 3 | 1 | 0 | 0 |
| ES | 1 | SI | Bona 12 | FD2 | 4 | 1 | 3 | 3 |
| ES | 1 | SI | Bona 12 | FD2 | 5 | 1 | 3 | 0 |
| ES | 1 | SI | Bona 12 | FD2 | 6 | 0 | 1 | 4 |
| ES | 1 | SI | Bona 12 | FD2 | 7 | 1 | 0 | 0 |
| ES | 1 | SI | Bona 12 | FD3 | 1 | 0 | 2 | 0 |
| ES | 1 | SI | Bona 12 | FD3 | 2 | 2 | 2 | 5 |
| ES | 1 | SI | Bona 12 | FD3 | 3 | 2 | 1 | 5 |
| ES | 1 | SI | Bona 12 | FD3 | 4 | 0 | 1 | 0 |
| ES | 1 | SI | Bona 12 | FD3 | 5 | 1 | 1 | 1 |
| ES | 1 | SI | Bona 12 | FD3 | 6 | 1 | 2 | 3 |
| ES | 1 | SI | Bona 12 | FD3 | 7 | 0 | 0 | 0 |
| ES | 1 | SI | Bona 12 | FD3 | 8 | 1 | 1 | 2 |
| ES | 1 | SI | Degumille 9 | FD1 | 1 | 5 | 5 | 2 |
| ES | 1 | SI | Degumille 9 | FD1 | 2 | 0 | 1 | 0 |
| ES | 1 | SI | Degumille 9 | FD1 | 3 | 2 | 4 | 4 |
| ES | 1 | SI | Degumille 9 | FD1 | 4 | 5 | 4 | 1 |
| ES | 1 | SI | Degumille 9 | FD1 | 5 | 5 | 5 | 4 |
| ES | 1 | SI | Degumille 9 | FD1 | 6 | 3 | 3 | 0 |
| ES | 1 | SI | Degumille 9 | FD2 | 1 | 0 | 0 | 5 |
| ES | 1 | SI | Degumille 9 | FD2 | 2 | 1 | 1 | 5 |
| ES | 1 | SI | Degumille 9 | FD2 | 3 | 1 | 2 | 5 |
| ES | 1 | SI | Degumille 9 | FD2 | 4 | 1 | 1 | 0 |
| ES | 1 | SI | Degumille 9 | FD2 | 5 | 1 | 1 | 5 |
| ES | 1 | SI | Degumille 9 | FD2 | 6 | 3 | 3 | 1 |
| ES | 1 | SI | Degumille 9 | FD3 | 1 | 1 | 2 | 4 |
| ES | 1 | SI | Degumille 9 | FD3 | 2 | 1 | 2 | 4 |
| ES | 1 | SI | Degumille 9 | FD3 | 3 | 1 | 1 | 1 |
| ES | 1 | SI | Degumille 9 | FD3 | 4 | 1 | 2 | 3 |
| ES | 1 | SI | Degumille 9 | FD3 | 5 | 0 | 1 | 3 |
| ES | 1 | SI | Degumille 9 | FD3 | 6 | 0 | 1 | 3 |
| ES | 1 | SI | Degumille 9 | FD3 | 7 | 1 | 5 | 5 |
| ES | 1 | SI | Degumille 44 | FD1 | 1 | 4 | 4 | 1 |
| ES | 1 | SI | Degumille 44 | FD1 | 2 | 4 | 2 | 0 |
| ES | 1 | SI | Degumille 44 | FD1 | 3 | 2 | 3 | 0 |
| ES | 1 | SI | Degumille 44 | FD1 | 4 | 3 | 4 | 3 |
| ES | 1 | SI | Degumille 44 | FD1 | 5 | 2 | 0 | 0 |
| ES | 1 | SI | Degumille 44 | FD1 | 6 | 5 | 3 | 0 |
| ES | 1 | SI | Degumille 44 | FD2 | 1 | 1 | 0 | 0 |
| ES | 1 | SI | Degumille 44 | FD2 | 2 | 0 | 0 | 2 |
| ES | 1 | SI | Degumille 44 | FD2 | 3 | 0 | 1 | 1 |
| ES | 1 | SI | Degumille 44 | FD2 | 4 | 0 | 1 | 4 |
| ES | 1 | SI | Degumille 44 | FD2 | 5 | 0 | 1 | 3 |
| ES | 1 | SI | Degumille 44 | FD2 | 6 | 0 | 1 | 1 |
| ES | 1 | SI | Degumille 44 | FD3 | 1 | 2 | 1 | 5 |
| ES | 1 | SI | Degumille 44 | FD3 | 2 | 1 | 3 | 4 |
| ES | 1 | SI | Degumille 44 | FD3 | 3 | 0 | 1 | 3 |
| ES | 1 | SI | Degumille 44 | FD3 | 4 | 1 | 4 | 5 |
| ES | 1 | SI | Degumille 44 | FD3 | 5 | 1 | 2 | 3 |
| ES | 1 | SI | Degumille 44 | FD3 | 6 | 0 | 0 | 1 |
| ES | 1 | SI | Degumille 44 | FD3 | 7 | 2 | 4 | 3 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

# Appendix A.3: Assessment list of test series *PE0*

**Table A.3:** Assessment list of test series *PE0*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| PE | 0 | SC | Bona 5 | FD1 | 1 | 1 | 1 | 0 |
| PE | 0 | SC | Bona 5 | FD1 | 2 | 0 | 0 | 0 |
| PE | 0 | SC | Bona 5 | FD1 | 3 | 1 | 2 | 3 |
| PE | 0 | SC | Bona 5 | FD1 | 4 | 0 | 0 | 0 |
| PE | 0 | SC | Bona 5 | FD1 | 5 | 1 | 2 | 2 |
| PE | 0 | SC | Bona 5 | FD2 | 1 | 2 | 3 | 3 |
| PE | 0 | SC | Bona 5 | FD2 | 2 | 3 | 3 | 3 |
| PE | 0 | SC | Bona 5 | FD2 | 3 | 2 | 3 | 3 |
| PE | 0 | SC | Bona 5 | FD2 | 4 | 4 | 4 | 4 |
| PE | 0 | SC | Bona 5 | FD2 | 5 | 4 | 4 | 4 |
| PE | 0 | SC | Bona 5 | FD2 | 6 | 3 | 3 | 3 |
| PE | 0 | SC | Bona 5 | FD2 | 7 | 4 | 4 | 4 |
| PE | 0 | SC | Bona 5 | FD3 | 1 | 4 | 4 | 5 |
| PE | 0 | SC | Bona 5 | FD3 | 2 | 3 | 4 | 3 |
| PE | 0 | SC | Bona 5 | FD3 | 3 | 2 | 3 | 4 |
| PE | 0 | SC | Bona 5 | FD3 | 4 | 3 | 3 | 5 |
| PE | 0 | SC | Bona 5 | FD3 | 5 | 4 | 4 | 3 |
| PE | 0 | SC | Bona 5 | FD3 | 6 | 3 | 4 | 4 |
| PE | 0 | SC | Bona 15 | FD1 | 1 | 1 | 1 | 0 |
| PE | 0 | SC | Bona 15 | FD1 | 2 | 2 | 1 | 0 |
| PE | 0 | SC | Bona 15 | FD1 | 3 | 2 | 3 | 1 |
| PE | 0 | SC | Bona 15 | FD1 | 4 | 3 | 2 | 0 |
| PE | 0 | SC | Bona 15 | FD1 | 5 | 1 | 1 | 0 |
| PE | 0 | SC | Bona 15 | FD1 | 6 | 1 | 1 | 0 |
| PE | 0 | SC | Bona 15 | FD1 | 7 | 0 | 1 | 1 |
| PE | 0 | SC | Bona 15 | FD1 | 8 | 1 | 1 | 0 |
| PE | 0 | SC | Bona 15 | FD1 | 9 | 2 | 2 | 1 |
| PE | 0 | SC | Bona 15 | FD1 | 10 | 0 | 0 | 1 |
| PE | 0 | SC | Bona 15 | FD2 | 1 | 1 | 2 | 0 |
| PE | 0 | SC | Bona 15 | FD2 | 2 | 1 | 1 | 0 |
| PE | 0 | SC | Bona 15 | FD2 | 3 | 1 | 1 | 2 |
| PE | 0 | SC | Bona 15 | FD2 | 4 | 1 | 2 | 0 |
| PE | 0 | SC | Bona 15 | FD2 | 5 | 0 | 1 | 0 |
| PE | 0 | SC | Bona 15 | FD2 | 6 | 2 | 3 | 0 |
| PE | 0 | SC | Bona 15 | FD3 | 1 | 3 | 4 | 4 |
| PE | 0 | SC | Bona 15 | FD3 | 2 | 4 | 4 | 5 |
| PE | 0 | SC | Bona 15 | FD3 | 3 | 4 | 4 | 4 |
| PE | 0 | SC | Bona 15 | FD3 | 4 | 4 | 5 | 5 |
| PE | 0 | SC | Bona 15 | FD3 | 5 | 5 | 5 | 4 |
| PE | 0 | SC | Bona 15 | FD3 | 6 | 3 | 4 | 4 |
| PE | 0 | SC | Degumille 27 | FD1 | 1 | 2 | 2 | 0 |
| PE | 0 | SC | Degumille 27 | FD1 | 2 | 1 | 2 | 1 |
| PE | 0 | SC | Degumille 27 | FD1 | 3 | 1 | 1 | 1 |
| PE | 0 | SC | Degumille 27 | FD1 | 4 | 1 | 2 | 1 |
| PE | 0 | SC | Degumille 27 | FD1 | 5 | 1 | 1 | 1 |
| PE | 0 | SC | Degumille 27 | FD1 | 6 | 0 | 2 | 1 |
| PE | 0 | SC | Degumille 27 | FD2 | 1 | 0 | 2 | 0 |
| PE | 0 | SC | Degumille 27 | FD2 | 2 | 3 | 3 | 2 |
| PE | 0 | SC | Degumille 27 | FD2 | 3 | 3 | 4 | 3 |
| PE | 0 | SC | Degumille 27 | FD2 | 4 | 3 | 4 | 3 |
| PE | 0 | SC | Degumille 27 | FD2 | 5 | 2 | 3 | 1 |
| PE | 0 | SC | Degumille 27 | FD2 | 6 | 2 | 3 | 3 |
| PE | 0 | SC | Degumille 27 | FD2 | 7 | 2 | 2 | 0 |
| PE | 0 | SC | Degumille 27 | FD2 | 8 | 2 | 3 | 2 |
| PE | 0 | SC | Degumille 27 | FD2 | 9 | 3 | 3 | 2 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

**Table A.3 (continued):** Assessment list of test series *PE0*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| PE | 0 | SC | Degumille 27 | FD3 | 1 | 5 | 5 | 5 |
| PE | 0 | SC | Degumille 27 | FD3 | 2 | 3 | 4 | 1 |
| PE | 0 | SC | Degumille 27 | FD3 | 3 | 4 | 4 | 1 |
| PE | 0 | SC | Degumille 27 | FD3 | 4 | 4 | 5 | 4 |
| PE | 0 | SC | Degumille 27 | FD3 | 5 | 4 | 5 | 0 |
| PE | 0 | SC | Degumille 27 | FD3 | 6 | 3 | 4 | 4 |
| PE | 0 | SI | Bona 7 | FD1 | 1 | 3 | 4 | 1 |
| PE | 0 | SI | Bona 7 | FD1 | 2 | 3 | 3 | 0 |
| PE | 0 | SI | Bona 7 | FD1 | 3 | 1 | 0 | 0 |
| PE | 0 | SI | Bona 7 | FD1 | 4 | 2 | 1 | 1 |
| PE | 0 | SI | Bona 7 | FD2 | 1 | 1 | 2 | 2 |
| PE | 0 | SI | Bona 7 | FD2 | 2 | 3 | 3 | 0 |
| PE | 0 | SI | Bona 7 | FD2 | 3 | 1 | 2 | 1 |
| PE | 0 | SI | Bona 7 | FD2 | 4 | 1 | 1 | 5 |
| PE | 0 | SI | Bona 7 | FD2 | 5 | 2 | 2 | 3 |
| PE | 0 | SI | Bona 7 | FD2 | 6 | 0 | 2 | 1 |
| PE | 0 | SI | Bona 7 | FD3 | 1 | 3 | 3 | 0 |
| PE | 0 | SI | Bona 7 | FD3 | 2 | 3 | 4 | 0 |
| PE | 0 | SI | Bona 7 | FD3 | 3 | 3 | 3 | 0 |
| PE | 0 | SI | Bona 12 | FD1 | 1 | 2 | 1 | 0 |
| PE | 0 | SI | Bona 12 | FD1 | 2 | 4 | 5 | 1 |
| PE | 0 | SI | Bona 12 | FD1 | 3 | 3 | 2 | 0 |
| PE | 0 | SI | Bona 12 | FD1 | 4 | 1 | 1 | 1 |
| PE | 0 | SI | Bona 12 | FD1 | 5 | 3 | 3 | 1 |
| PE | 0 | SI | Bona 12 | FD1 | 6 | 3 | 3 | 3 |
| PE | 0 | SI | Bona 12 | FD1 | 7 | 4 | 5 | 5 |
| PE | 0 | SI | Bona 12 | FD2 | 1 | 4 | 4 | 1 |
| PE | 0 | SI | Bona 12 | FD2 | 2 | 3 | 3 | 1 |
| PE | 0 | SI | Bona 12 | FD2 | 3 | 3 | 3 | 1 |
| PE | 0 | SI | Bona 12 | FD2 | 4 | 4 | 4 | 0 |
| PE | 0 | SI | Bona 12 | FD2 | 5 | 3 | 4 | 0 |
| PE | 0 | SI | Bona 12 | FD2 | 6 | 2 | 2 | 1 |
| PE | 0 | SI | Bona 12 | FD2 | 7 | 2 | 2 | 1 |
| PE | 0 | SI | Bona 12 | FD3 | 1 | 4 | 3 | 2 |
| PE | 0 | SI | Bona 12 | FD3 | 2 | 4 | 4 | 2 |
| PE | 0 | SI | Bona 12 | FD3 | 3 | 3 | 3 | 1 |
| PE | 0 | SI | Bona 12 | FD3 | 4 | 3 | 4 | 2 |
| PE | 0 | SI | Bona 12 | FD3 | 5 | 5 | 5 | 3 |
| PE | 0 | SI | Bona 12 | FD3 | 6 | 5 | 4 | 4 |
| PE | 0 | SI | Bona 12 | FD3 | 7 | 5 | 5 | 4 |
| PE | 0 | SI | Bona 12 | FD3 | 8 | 5 | 5 | 3 |
| PE | 0 | SI | Degumille 8 | FD1 | 1 | 0 | 0 | 0 |
| PE | 0 | SI | Degumille 8 | FD1 | 2 | 2 | 3 | 2 |
| PE | 0 | SI | Degumille 8 | FD1 | 3 | 0 | 0 | 0 |
| PE | 0 | SI | Degumille 8 | FD1 | 4 | 2 | 3 | 0 |
| PE | 0 | SI | Degumille 8 | FD1 | 5 | 0 | 1 | 0 |
| PE | 0 | SI | Degumille 8 | FD1 | 6 | 0 | 1 | 0 |
| PE | 0 | SI | Degumille 8 | FD2 | 1 | 3 | 3 | 0 |
| PE | 0 | SI | Degumille 8 | FD2 | 2 | 3 | 3 | 0 |
| PE | 0 | SI | Degumille 8 | FD2 | 3 | 2 | 3 | 0 |
| PE | 0 | SI | Degumille 8 | FD2 | 4 | 3 | 3 | 0 |
| PE | 0 | SI | Degumille 8 | FD2 | 5 | 3 | 3 | 1 |
| PE | 0 | SI | Degumille 8 | FD2 | 6 | 4 | 5 | 1 |
| PE | 0 | SI | Degumille 8 | FD2 | 7 | 2 | 2 | 0 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

**Table A.3 (continued):** Assessment list of test series *PE0*

| test | isolation | compatibility | genotype | stage | replication | fp | p | fi |
|---|---|---|---|---|---|---|---|---|
| PE | 0 | SI | Degumille 8 | FD3 | 1 | 2 | 5 | 0 |
| PE | 0 | SI | Degumille 8 | FD3 | 2 | 3 | 4 | 0 |
| PE | 0 | SI | Degumille 8 | FD3 | 3 | 3 | 5 | 1 |
| PE | 0 | SI | Degumille 8 | FD3 | 4 | 4 | 5 | 0 |
| PE | 0 | SI | Degumille 8 | FD3 | 5 | 4 | 4 | 0 |
| PE | 0 | SI | Degumille 8 | FD3 | 6 | 4 | 5 | 1 |
| PE | 0 | SI | Degumille 8 | FD3 | 7 | 1 | 3 | 5 |
| PE | 0 | SI | Degumille 9 | FD1 | 1 | 0 | 2 | 2 |
| PE | 0 | SI | Degumille 9 | FD1 | 2 | 1 | 4 | 1 |
| PE | 0 | SI | Degumille 9 | FD1 | 3 | 2 | 3 | 3 |
| PE | 0 | SI | Degumille 9 | FD1 | 4 | 2 | 5 | 4 |
| PE | 0 | SI | Degumille 9 | FD1 | 5 | 1 | 2 | 2 |
| PE | 0 | SI | Degumille 9 | FD1 | 6 | | | 1 |
| PE | 0 | SI | Degumille 9 | FD1 | 7 | 2 | 5 | 3 |
| PE | 0 | SI | Degumille 9 | FD2 | 1 | 1 | 2 | 4 |
| PE | 0 | SI | Degumille 9 | FD2 | 2 | 0 | 2 | 4 |
| PE | 0 | SI | Degumille 9 | FD2 | 3 | 2 | 1 | 2 |
| PE | 0 | SI | Degumille 9 | FD2 | 4 | 0 | 2 | 2 |
| PE | 0 | SI | Degumille 9 | FD2 | 5 | 0 | 1 | 0 |
| PE | 0 | SI | Degumille 9 | FD2 | 6 | 0 | 1 | 0 |
| PE | 0 | SI | Degumille 9 | FD2 | 7 | 2 | 2 | 4 |
| PE | 0 | SI | Degumille 9 | FD3 | 1 | 2 | 5 | 1 |
| PE | 0 | SI | Degumille 9 | FD3 | 2 | 2 | 5 | 3 |
| PE | 0 | SI | Degumille 9 | FD3 | 3 | 2 | 4 | 5 |
| PE | 0 | SI | Degumille 9 | FD3 | 4 | 1 | 4 | 4 |
| PE | 0 | SI | Degumille 9 | FD3 | 5 | 2 | 3 | 2 |
| PE | 0 | SI | Degumille 9 | FD3 | 6 | 2 | 5 | 0 |
| PE | 0 | SI | Degumille 9 | FD3 | 7 | 3 | 5 | 4 |

Assessment for papillae (p), pseudo-papillae (fp) and filaments (fi). Degrees of receptivity: (0) no reaction; (1) very low reaction; (2) weak positive reaction; (3) medium positive reaction; (4) strong positive reaction; and (5) very strong positive reaction.

# Appendix B: Analysis of variance

## Appendix B.1: Variance analysis of test series *ES0*

**Table B.1:** Variance analysis of test series *ES0*

**Papillae**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr (>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 2.92 | 2.923 | 1.531 | 0.21898 | |
| genotype | 6 | 35.52 | 5.920 | 3.101 | 0.00807 | ** |
| stage | 2 | 23.03 | 11.517 | 6.033 | 0.00340 | ** |
| compatibility and stage | 2 | 0.40 | 0.200 | 0.105 | 0.90049 | |
| genotype and stage | 12 | 72.76 | 6.064 | 3.177 | 0.00075 | *** |
| residuals | 96 | 183.26 | 1.909 | | | |

**Pseudo-Papillae**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr (>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 0.08 | 0.082 | 0.060 | 0.807230 | |
| genotype | 6 | 28.76 | 4.793 | 3.503 | 0.003546 | ** |
| stage | 2 | 31.88 | 15.940 | 11.651 | 2.95e-05 | *** |
| compatibility and stage | 2 | 4.55 | 2.273 | 1.661 | 0.195359 | |
| genotype and stage | 12 | 53.76 | 4.480 | 3.274 | 0.000546 | *** |
| residuals | 96 | 131.34 | 1.368 | | | |

**Filaments**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr (>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 0.01 | 0.011 | 0.004 | 0.951933 | |
| genotype | 6 | 79.72 | 13.287 | 4.529 | 0.000438 | *** |
| stage | 2 | 44.78 | 22.390 | 7.632 | 0.000839 | *** |
| compatibility and stage | 2 | 19.46 | 9.728 | 3.316 | 0.040501 | * |
| genotype and stage | 12 | 42.77 | 3.565 | 1.215 | 0.284117 | |
| residuals | 96 | 281.62 | 2.934 | | | |

One-way analysis of variance. (Df) Degrees of freedom. (Sum Sq) Sum of squares. (Mean Sq) Mean of squares. (Pr (>F)) Probability value. Significance level $\alpha = 0{,}05$. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ' and 1.

# Appendix B.2: Variance analysis of test series *ES1*

**Table B.2:** Variance analysis of test series *ES1*

**Papillae**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr(>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 13.24 | 13.242 | 8.213 | 0.004846 | ** |
| genotype | 6 | 18.38 | 3.063 | 1.900 | 0.085519 | . |
| stage | 2 | 9.63 | 4.814 | 2.986 | 0.053941 | . |
| compatibility and stage | 2 | 56.22 | 28.110 | 17.436 | 1.93e-07 | *** |
| genotype and stage | 12 | 63.08 | 5.257 | 3.261 | 0.000389 | *** |
| residuals | 131 | 211.20 | 1.612 | | | |

**Pseudo-Papillae**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr(>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 4.69 | 4.69 | 3.960 | 0.048625 | * |
| genotype | 6 | 29.96 | 4.99 | 4.217 | 0.000641 | *** |
| stage | 2 | 94.22 | 47.11 | 39.792 | 2.72e-14 | *** |
| compatibility and stage | 2 | 38.11 | 19.05 | 16.094 | 5.44e-07 | *** |
| genotype and stage | 12 | 41.55 | 3.46 | 2.925 | 0.001242 | ** |
| residuals | 134 | 158.65 | 1.18 | | | |

**Filaments**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr(>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 0.6 | 0.62 | 0.198 | 0.6568 | |
| genotype | 6 | 118.8 | 19.80 | 6.373 | 6.21e-06 | *** |
| stage | 2 | 92.4 | 46.21 | 14.872 | 1.44e-06 | *** |
| compatibility and stage | 2 | 8.8 | 4.41 | 1.418 | 0.2458 | |
| genotype and stage | 12 | 60.2 | 5.02 | 1.614 | 0.0944 | . |
| residuals | 134 | 422.6 | 3.11 | | | |

One-way analysis of variance. (Df) Degrees of freedom. (Sum Sq) Sum of squares. (Mean Sq) Mean of squares. (Pr (>F)) Probability value. Significance level $\alpha = 0{,}05$. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ' and 1.

# Appendix B.3: Variance analysis of test series *PE0*

**Table B.3:** Variance analysis of test series *PE0*

**Papillae**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr (>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 3.96 | 3.96 | 5.438 | 0.021445 | * |
| genotype | 5 | 16.64 | 3.33 | 4.568 | 0.000775 | *** |
| stage | 2 | 105.23 | 52.62 | 72.223 | < 2e-16 | *** |
| compatibility and stage | 2 | 11.48 | 5.74 | 7.877 | 0.000622 | *** |
| genotype stage | 10 | 40.79 | 4.08 | 5.600 | 8.9e-07 | *** |
| residuals | 115 | 83.78 | 0.73 | | | |

**Pseudo-Papillae**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr (>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 0.06 | 0.06 | 0.089 | 0.76569 | |
| genotype | 5 | 43.68 | 8.74 | 12.755 | 7.25e-10 | *** |
| stage | 2 | 74.53 | 37.26 | 54.414 | < 2e-16 | *** |
| compatibility and stage | 2 | 8.99 | 4.50 | 6.566 | 0.00199 | ** |
| genotype and stage | 10 | 31.59 | 3.16 | 4.613 | 1.63e-05 | *** |
| residuals | 115 | 78.76 | 0.68 | | | |

**Filaments**

| Factor | Df | Sum Sq | Mean Sq | F value | Pr (>F) | Signif. |
|---|---|---|---|---|---|---|
| compatibility | 1 | 8.35 | 8.351 | 5.641 | 0.019190 | * |
| genotype | 5 | 63.50 | 12.699 | 8.578 | 6.12e-07 | *** |
| stage | 2 | 55.94 | 27.972 | 18.894 | 7.89e-08 | *** |
| compatibility and stage | 2 | 26.48 | 13.239 | 8.943 | 0.000244 | *** |
| genotype and stage | 10 | 48.32 | 4.832 | 3.264 | 0.000952 | *** |
| residuals | 116 | 171.74 | 1.480 | | | |

One-way analysis of variance. (Df) Degrees of freedom. (Sum Sq) Sum of squares. (Mean Sq) Mean of squares. (Pr (>F)) Probability value. Significance level $\alpha = 0{,}05$. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ' and 1.

# Appendix C: Post-hoc comparisons

The following tables show the results of the post-hoc comparisons, all of which are based on Tukey's HSD test, giving the difference between the mean values of the two groups (diff), the lower end point of the interval (lwr), the upper end point (upr) and the p-value (p adj), with the significance level: $\alpha = 0{,}05$; designated as statistically not significant: $p \geq 0{,}05$ [ ]; statistically significant: $p < 0{,}05$ [*]; statistically highly significant: $p < 0{,}01$ [**], $p < 0{,}001$ [***].

## Appendix C.1: Post-hoc comparisons of test series *ES0*

**Table C.1: Comparison between the individual stages of floral development (*ES0*)**

| **Papillae** | | | | |
|---|---|---|---|---|
| Comparison | diff | lwr | upr | p adj |
| FD2-FD1 | - 0.03764706 | - 0.87434539 | 0.7990513 | 0.9937300 |
| FD3-FD1 | 0.95500000 | 0.13225227 | 1.7777477 | 0.0185151* |
| FD3-FD2 | 0.99264706 | 0.09250298 | 1.8927911 | 0.0268792* |
| **Pseudo-Papillae** | | | | |
| Comparison | diff | lwr | upr | p adj |
| FD2-FD1 | - 1.2241176 | - 1.9432743 | - 0.5049610 | 0.0002804*** |
| FD3-FD1 | - 0.1661111 | - 0.8732770 | 0.5410547 | 0.8427635 |
| FD3-FD2 | 1.0580065 | 0.2843172 | 1.8316959 | 0.0043216** |
| **Filament** | | | | |
| Comparison | diff | lwr | upr | p adj |
| FD2-FD1 | 1.41536615 | 0.4185553 | 2.4121769 | 0.0029009** |
| FD3-FD1 | 1.32156646 | 0.3489054 | 2.2942275 | 0.0046145** |
| FD3-FD2 | - 0.09379968 | - 1.1547622 | 0.9671628 | 0.9760144 |

Mean esterase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) compared between the individual stages of floral development (FD1, FD2, FD3).

**Table C.2: Comparison between self-compatible and self-incompatible genotypes (*ES0*)**

| SI-SC | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| diff | 0.3122914 | - 0.05228031 | 0.0189099 |
| lwr | - 0.2787297 | - 0.57912410 | - 0.7017871 |
| upr | 0.9033125 | 0.47456350 | 0.7396069 |
| p adj | 0.2975326 | 0.84455030 | 0.9586492 |

Mean esterase activity compared between self-compatible (SC) and self-incompatible (SI) genotypes.

**Table C.3: Comparison between SC and SI genotypes at different stages of floral development (*ES0*)**

| **Papillae** | | | | |
|---|---|---|---|---|
| SI-SC | diff | lwr | upr | p adj |
| FD1 | 0.4758454 | - 0.8378956 | 1.7895864 | 0.8997609 |
| FD2 | 0.1631944 | - 1.4275989 | 1.7539878 | 0.9996804 |
| FD3 | 0.1811146 | - 1.3645689 | 1.7267980 | 0.9993882 |
| **Pseudo-Papillae** | | | | |
| SI-SC | diff | lwr | upr | p adj |
| FD1 | 0.31320451 | - 0.8102355 | 1.43664447 | 0.9655075 |
| FD2 | - 0.27083333 | - 1.6311934 | 1.08952675 | 0.9923456 |
| FD3 | - 0.55882353 | - 1.8806080 | 0.76296097 | 0.8235049 |
| **Filament** | | | | |
| SI-SC | diff | lwr | upr | p adj |
| FD1 | 0.9331104 | - 0.61639196 | 2.4826127 | 0.5048876 |
| FD2 | - 0.3750000 | - 2.23489317 | 1.4848932 | 0.9918848 |
| FD3 | - 0.7176471 | - 2.50333756 | 1.0680434 | 0.8524391 |

Mean esterase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) compared between self-compatible (SC) and self-incompatible (SI) genotypes at different floral development stages (FD1, FD2, FD3).

**Table C.4: Esterase activity of papillae compared between genotypes (*ES0*)**

| Comparison (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 0.8850000 | -0.5835253 | 2.3535253 | 0.5762751 |
| Bona5-Bona12 | -0.8226085 | -2.4000577 | 0.7548408 | 0.7395946 |
| Bona7-Bona12 | -0.2045848 | -1.6731101 | 1.2639406 | 0.9998639 |
| Degumille27-Bona12 | -0.7067431 | -2.1980376 | 0.7845515 | 0.8219958 |
| Degumille8-Bona12 | 0.0379523 | -1.5072426 | 1.5831472 | 1.0000000 |
| Degumille9-Bona12 | 0.5827842 | -0.9624107 | 2.1279792 | 0.9387978 |
| Degumille97-Bona12 | -0.4522088 | -2.0664737 | 1.1620562 | 0.9881855 |
| Bona5-Bona15 | -1.7076085 | -3.2850577 | -0.1301592 | 0.0242436* |
| Bona7-Bona15 | -1.0895848 | -2.5581101 | 0.3789406 | 0.3049159 |
| Degumille27-Bona15 | -1.5917431 | -3.0830376 | -0.1004485 | 0.0278200* |
| Degumille8-Bona15 | -0.8470477 | -2.3922426 | 0.6981472 | 0.6880455 |
| Degumille9-Bona15 | -0.3022158 | -1.8474107 | 1.2429792 | 0.9987259 |
| Degumille97-Bona15 | -1.3372088 | -2.9514737 | 0.2770562 | 0.1811214 |
| Bona7-Bona5 | 0.6180237 | -0.9594256 | 2.1954730 | 0.9258675 |
| Degumille27-Bona5 | 0.1158654 | -1.4828025 | 1.7145332 | 0.9999984 |
| Degumille8-Bona5 | 0.8605608 | -0.7885017 | 2.5096232 | 0.7389055 |
| Degumille9-Bona5 | 1.4053927 | -0.2436697 | 3.0544551 | 0.1543901 |
| Degumille97-Bona5 | 0.3703997 | -1.3435521 | 2.0843515 | 0.9975904 |
| Degumille27-Bona7 | -0.5021583 | -1.9934528 | 0.9891362 | 0.9663738 |
| Degumille8-Bona7 | 0.2425371 | -1.3026579 | 1.7877320 | 0.9996982 |
| Degumille9-Bona7 | 0.7873690 | -0.7578259 | 2.3325639 | 0.7615124 |
| Degumille97-Bona7 | -0.2476240 | -1.8618890 | 1.3666410 | 0.9997409 |
| Degumille8-Degumille27 | 0.7446954 | -0.8221550 | 2.3115458 | 0.8198277 |
| Degumille9-Degumille27 | 1.2895273 | -0.2773231 | 2.8563777 | 0.1875676 |
| Degumille97-Degumille27 | 0.2545343 | -1.3804715 | 1.8895402 | 0.9997142 |
| Degumille9-Degumille8 | 0.5448319 | -1.0734042 | 2.1630680 | 0.9663977 |
| Degumille97-Degumille8 | -0.4901611 | -2.1744746 | 1.1941525 | 0.9852315 |
| Degumille97-Degumille9 | -1.0349930 | -2.7193065 | 0.6493206 | 0.5515129 |

Mean esterase activity of papillae (p) compared between the individual genotypes.

**Table C.5: Esterase activity of pseudo-papillae compared between genotypes (*ES0*)**

| Comparison (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 1.50977124 | 0.2665290 | 2.7530135 | 0.0067526** |
| Bona5-Bona12 | 0.53441901 | -0.8010374 | 1.8698754 | 0.9176987 |
| Bona7-Bona12 | 1.04858708 | -0.1946552 | 2.2918294 | 0.1638878 |
| Degumille27-Bona12 | 0.73786452 | -0.5246540 | 2.0003830 | 0.6142189 |
| Degumille8-Bona12 | 0.98224186 | -0.3259083 | 2.2903920 | 0.2902226 |
| Degumille9-Bona12 | 1.48626613 | 0.1781160 | 2.7944163 | 0.0146611* |
| Degumille97-Bona12 | 0.93345957 | -0.4331648 | 2.3000839 | 0.4119564 |
| Bona5-Bona15 | -0.97535223 | -2.3108087 | 0.3601042 | 0.3246958 |
| Bona7-Bona15 | -0.46118416 | -1.7044264 | 0.7820581 | 0.9437913 |
| Degumille27-Bona15 | -0.77190672 | -2.0344252 | 0.4906118 | 0.5579661 |
| Degumille8-Bona15 | -0.52752938 | -1.8356796 | 0.7806208 | 0.9145535 |
| Degumille9-Bona15 | -0.02350511 | -1.3316553 | 1.2846451 | 1.0000000 |
| Degumille97-Bona15 | -0.57631167 | -1.9429360 | 0.7903127 | 0.8941795 |
| Bona7-Bona5 | 0.51416807 | -0.8212884 | 1.8496245 | 0.9320603 |
| Degumille27-Bona5 | 0.20344551 | -1.1499744 | 1.5568654 | 0.9997737 |
| Degumille8-Bona5 | 0.44782285 | -0.9482607 | 1.8439064 | 0.9742740 |
| Degumille9-Bona5 | 0.95184712 | -0.4442365 | 2.3479307 | 0.4143740 |
| Degumille97-Bona5 | 0.39904056 | -1.0519779 | 1.8500590 | 0.9894079 |
| Degumille27-Bona7 | -0.31072256 | -1.5732411 | 0.9517959 | 0.9945809 |
| Degumille8-Bona7 | -0.06634522 | -1.3744954 | 1.2418049 | 0.9999999 |
| Degumille9-Bona7 | 0.43767905 | -0.8704711 | 1.7458292 | 0.9675283 |
| Degumille97-Bona7 | -0.11512752 | -1.4817518 | 1.2514968 | 0.9999956 |
| Degumille8-Degumille27 | 0.24437733 | -1.0821062 | 1.5708608 | 0.9991350 |
| Degumille9-Degumille27 | 0.74840161 | -0.5780819 | 2.0748851 | 0.6558386 |
| Degumille97-Degumille27 | 0.19559504 | -1.1885883 | 1.5797784 | 0.9998503 |
| Degumille9-Degumille8 | 0.50402428 | -0.8659620 | 1.8740105 | 0.9461387 |
| Degumille97-Degumille8 | -0.04878229 | -1.4747092 | 1.3771446 | 1.0000000 |
| Degumille97-Degumille9 | -0.55280657 | -1.9787335 | 0.8731203 | 0.9296625 |

Mean esterase activity of pseudo-papillae (fp) compared between the individual genotypes.

**Table C.6: Esterase activity of filaments compared between genotypes (*ES0*)**

| Comparison (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 0.73823520 | -1.0567895 | 2.5332599 | 0.9060446 |
| Bona5-Bona12 | -0.36018025 | -2.2920153 | 1.5716548 | 0.9990651 |
| Bona7-Bona12 | 1.00899788 | -0.7860268 | 2.8040225 | 0.6600874 |
| Degumille27-Bona12 | 1.42730809 | -0.3963370 | 3.2509532 | 0.2413127 |
| Degumille8-Bona12 | 0.57780178 | -1.3135479 | 2.4691515 | 0.9804651 |
| Degumille9-Bona12 | 2.27544032 | 0.3436053 | 4.2072753 | 0.0097552** |
| Degumille97-Bona12 | 1.45435742 | -0.5236634 | 3.4323783 | 0.3162475 |
| Bona5-Bona15 | -1.09841545 | -3.0539325 | 0.8571016 | 0.6609134 |
| Bona7-Bona15 | 0.27076268 | -1.5497246 | 2.0912500 | 0.9997891 |
| Degumille27-Bona15 | 0.68907289 | -1.1596407 | 2.5377865 | 0.9423900 |
| Degumille8-Bona15 | -0.16043342 | -2.0759657 | 1.7550989 | 0.9999958 |
| Degumille9-Bona15 | 1.53720512 | -0.4183119 | 3.4927222 | 0.2363958 |
| Degumille97-Bona15 | 0.71612222 | -1.2850342 | 2.7172786 | 0.9533993 |
| Bona7-Bona5 | 1.36917812 | -0.5863389 | 3.3246952 | 0.3792437 |
| Degumille27-Bona5 | 1.78748833 | -0.1943328 | 3.7693094 | 0.1084366 |
| Degumille8-Bona5 | 0.93798202 | -1.1063117 | 2.9822758 | 0.8449837 |
| Degumille9-Bona5 | 2.63562057 | 0.5538137 | 4.7174274 | 0.0039464** |
| Degumille97-Bona5 | 1.81453767 | -0.3101976 | 3.9392729 | 0.1525336 |
| Degumille27-Bona7 | 0.41831021 | -1.4304034 | 2.2670238 | 0.9967821 |
| Degumille8-Bona7 | -0.43119610 | -2.3467284 | 1.4843362 | 0.9968846 |
| Degumille9-Bona7 | 1.26644245 | -0.6890746 | 3.2219595 | 0.4827998 |
| Degumille97-Bona7 | 0.44535954 | -1.5557968 | 2.4465159 | 0.9971000 |
| Degumille8-Degumille27 | -0.84950631 | -2.7918842 | 1.0928716 | 0.8747595 |
| Degumille9-Degumille27 | 0.84813224 | -1.1336889 | 2.8299533 | 0.8866555 |
| Degumille97-Degumille27 | 0.02704933 | -1.9998189 | 2.0539176 | 1.0000000 |
| Degumille9-Degumille8 | 1.69763854 | -0.3466552 | 3.7419323 | 0.1786915 |
| Degumille97-Degumille8 | 0.87655564 | -1.2114379 | 2.9645492 | 0.8964007 |
| Degumille97-Degumille9 | -0.82108290 | -2.9458181 | 1.3036523 | 0.9307852 |

Mean esterase activity of filaments (fi) compared between the individual genotypes.

**Table C.7: Esterase activity of papillae compared between genotypes in stage FD1 (*ES0*)**

| Comparison FD1 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 1.678571e+00 | -1.20041901 | 4.55756186 | 0.8679424 |
| Bona5-Bona12 | 6.785714e-01 | -2.20041901 | 3.55756186 | 0.9999997 |
| Bona7-Bona12 | 2.357143e+00 | -0.40890168 | 5.12318739 | 0.2117521 |
| Degumille27-Bona12 | -1.428571e-01 | -2.90890168 | 2.62318739 | 1.0000000 |
| Degumille8-Bona12 | 1.116071e+00 | -1.56213968 | 3.79428254 | 0.9964283 |
| Degumille9-Bona12 | 2.028571e+00 | -1.00147855 | 5.05862141 | 0.6675630 |
| Degumille97-Bona12 | 1.553571e+00 | -1.68990329 | 4.79704615 | 0.9800168 |
| Bona5-Bona15 | -1.000000e+00 | -3.98766957 | 1.98766957 | 0.9998634 |
| Bona7-Bona15 | 6.785714e-01 | -2.20041901 | 3.55756186 | 0.9999997 |
| Degumille27-Bona15 | -1.821429e+00 | -4.70041901 | 1.05756186 | 0.7627299 |
| Degumille8-Bona15 | -5.625000e-01 | -3.35720898 | 2.23220898 | 1.0000000 |
| Degumille9-Bona15 | 3.500000e-01 | -2.78349428 | 3.48349428 | 1.0000000 |
| Degumille97-Bona15 | -1.250000e-01 | -3.46531612 | 3.21531612 | 1.0000000 |
| Bona7-Bona5 | 1.678571e+00 | -1.20041901 | 4.55756186 | 0.8679424 |
| Degumille27-Bona5 | -8.214286e-01 | -3.70041901 | 2.05756186 | 0.9999911 |
| Degumille8-Bona5 | 4.375000e-01 | -2.35720898 | 3.23220898 | 1.0000000 |
| Degumille9-Bona5 | 1.350000e+00 | -1.78349428 | 4.48349428 | 0.9944618 |
| Degumille97-Bona5 | 8.750000e-01 | -2.46531612 | 4.21531612 | 0.9999981 |
| Degumille27-Bona7 | -2.500000e+00 | -5.26604454 | 0.26604454 | 0.1338315 |
| Degumille8-Bona7 | -1.241071e+00 | -3.91928254 | 1.43713968 | 0.9863575 |
| Degumille9-Bona7 | -3.285714e-01 | -3.35862141 | 2.70147855 | 1.0000000 |
| Degumille97-Bona7 | -8.035714e-01 | -4.04704615 | 2.43990329 | 0.9999993 |
| Degumille8-Degumille27 | 1.258929e+00 | -1.41928254 | 3.93713968 | 0.9838783 |
| Degumille9-Degumille27 | 2.171429e+00 | -0.85862141 | 5.20147855 | 0.5359483 |
| Degumille97-Degumille27 | 1.696429e+00 | -1.54704615 | 4.93990329 | 0.9495028 |
| Degumille9-Degumille8 | 9.125000e-01 | -2.03758732 | 3.86258732 | 0.9999633 |
| Degumille97-Degumille8 | 4.375000e-01 | -2.73140212 | 3.60640212 | 1.0000000 |

Mean esterase activity of papillae (p) compared between the individual genotypes in stage FD1.

**Table C.8: Esterase activity of papillae compared between genotypes in stage FD2 (*ES0*)**

| Comparison FD2 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -2.500000e-01 | -3.23766957 | 2.73766957 | 1.0000000 |
| Bona5-Bona12 | -2.333333e+00 | -5.99246631 | 1.32579965 | 0.7504704 |
| Bona7-Bona12 | -2.000000e+00 | -5.65913298 | 1.65913298 | 0.9235577 |
| Degumille27-Bona12 | -1.233333e+00 | -4.36682761 | 1.90016095 | 0.9983780 |
| Degumille8-Bona12 | -8.333333e-01 | -4.49246631 | 2.82579965 | 0.9999999 |
| Degumille9-Bona12 | -2.333333e+00 | -5.67364946 | 1.00698279 | 0.5869043 |
| Degumille97-Bona12 | -2.083333e+00 | -5.42364946 | 1.25698279 | 0.7840040 |
| Bona5-Bona15 | -2.083333e+00 | -5.74246631 | 1.57579965 | 0.8910772 |
| Bona7-Bona15 | -1.750000e+00 | -5.40913298 | 1.90913298 | 0.9803569 |
| Degumille27-Bona15 | -9.833333e-01 | -4.11682761 | 2.15016095 | 0.9999531 |
| Degumille8-Bona15 | -5.833333e-01 | -4.24246631 | 3.07579965 | 1.0000000 |
| Degumille9-Bona15 | -2.083333e+00 | -5.42364946 | 1.25698279 | 0.7840040 |
| Degumille97-Bona15 | -1.833333e+00 | -5.17364946 | 1.50698279 | 0.9206504 |
| Bona7-Bona5 | 3.333333e-01 | -3.89186949 | 4.55853616 | 1.0000000 |
| Degumille27-Bona5 | 1.100000e+00 | -2.67913629 | 4.87913629 | 0.9999873 |
| Degumille8-Bona5 | 1.500000e+00 | -2.72520282 | 5.72520282 | 0.9996514 |
| Degumille9-Bona5 | -8.881784e-16 | -3.95231534 | 3.95231534 | 1.0000000 |
| Degumille97-Bona5 | 2.500000e-01 | -3.70231534 | 4.20231534 | 1.0000000 |
| Degumille27-Bona7 | 7.666667e-01 | -3.01246963 | 4.54580296 | 1.0000000 |
| Degumille8-Bona7 | 1.166667e+00 | -3.05853616 | 5.39186949 | 0.9999950 |
| Degumille9-Bona7 | -3.333333e-01 | -4.28564867 | 3.61898201 | 1.0000000 |
| Degumille97-Bona7 | -8.333333e-02 | -4.03564867 | 3.86898201 | 1.0000000 |
| Degumille8-Degumille27 | 4.000000e-01 | -3.37913629 | 4.17913629 | 1.0000000 |
| Degumille9-Degumille27 | -1.100000e+00 | -4.57135834 | 2.37135834 | 0.9999448 |
| Degumille97-Degumille27 | -8.500000e-01 | -4.32135834 | 2.62135834 | 0.9999995 |

Mean esterase activity of papillae (p) compared between the individual genotypes in stage FD2.

**Table C.9: Esterase activity of papillae compared between genotypes in stage FD3 (*ES0*)**

| Comparison FD3 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 1.000000e+00 | -2.47135834 | 4.47135834 | 0.9999894 |
| Bona5-Bona12 | -1.375000e+00 | -5.03413298 | 2.28413298 | 0.9991730 |
| Bona7-Bona12 | -1.642857e+00 | -4.88633187 | 1.60061758 | 0.9634444 |
| Degumille27-Bona12 | -7.500000e-01 | -4.40913298 | 2.90913298 | 1.0000000 |
| Degumille8-Bona12 | -3.333333e-01 | -4.28564867 | 3.61898201 | 1.0000000 |
| Degumille9-Bona12 | 1.400000e+00 | -2.07135834 | 4.87135834 | 0.9977158 |
| Degumille97-Bona12 | -1.125000e+00 | -4.78413298 | 2.53413298 | 0.9999669 |
| Bona5-Bona15 | -2.375000e+00 | -5.84635834 | 1.09635834 | 0.6271384 |
| Bona7-Bona15 | -2.642857e+00 | -5.67290712 | 0.38719283 | 0.1783280 |
| Degumille27-Bona15 | -1.750000e+00 | -5.22135834 | 1.72135834 | 0.9651930 |
| Degumille8-Bona15 | -1.333333e+00 | -5.11246963 | 2.44580296 | 0.9996836 |
| Degumille9-Bona15 | 4.000000e-01 | -2.87282803 | 3.67282803 | 1.0000000 |
| Degumille97-Bona15 | -2.125000e+00 | -5.59635834 | 1.34635834 | 0.8100137 |
| Bona7-Bona5 | -2.678571e-01 | -3.51133187 | 2.97561758 | 1.0000000 |
| Degumille27-Bona5 | 6.250000e-01 | -3.03413298 | 4.28413298 | 1.0000000 |
| Degumille8-Bona5 | 1.041667e+00 | -2.91064867 | 4.99398201 | 0.9999979 |
| Degumille9-Bona5 | 2.775000e+00 | -0.69635834 | 6.24635834 | 0.3195957 |
| Degumille97-Bona5 | 2.500000e-01 | -3.40913298 | 3.90913298 | 1.0000000 |
| Degumille27-Bona7 | 8.928571e-01 | -2.35061758 | 4.13633187 | 0.9999953 |
| Degumille8-Bona7 | 1.309524e+00 | -2.26142433 | 4.88047195 | 0.9994264 |
| Degumille9-Bona7 | 3.042857e+00 | 0.01280717 | 6.07290712 | 0.0477152* |
| Degumille97-Bona7 | 5.178571e-01 | -2.72561758 | 3.76133187 | 1.0000000 |
| Degumille8-Degumille27 | 4.166667e-01 | -3.53564867 | 4.36898201 | 1.0000000 |
| Degumille9-Degumille27 | 2.150000e+00 | -1.32135834 | 5.62135834 | 0.7939708 |
| Degumille97-Degumille27 | -3.750000e-01 | -4.03413298 | 3.28413298 | 1.0000000 |

Mean esterase activity of papillae (p) compared between the individual genotypes in stage FD3.

**Table C.10: Esterase activity of pseudo-papillae compared between genotypes in stage FD1 (*ES0*)**

| Comparison FD1 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 1.845238e+00 | -0.59209309 | 4.28256928 | 0.4249151 |
| Bona5-Bona12 | 1.178571e+00 | -1.25875976 | 3.61590262 | 0.9777781 |
| Bona7-Bona12 | 2.142857e+00 | -0.19885492 | 4.48456920 | 0.1202616 |
| Degumille27-Bona12 | -2.142857e-01 | -2.55599778 | 2.12742635 | 1.0000000 |
| Degumille8-Bona12 | 1.491071e+00 | -0.77628152 | 3.75842438 | 0.6993636 |
| Degumille9-Bona12 | 2.728571e+00 | 0.16335439 | 5.29378847 | 0.0240296* |
| Degumille97-Bona12 | 2.678571e+00 | -0.06732936 | 5.42447222 | 0.0652231 |
| Bona5-Bona15 | -6.666667e-01 | -3.19600478 | 1.86267144 | 0.9999979 |
| Bona7-Bona15 | 2.976190e-01 | -2.13971214 | 2.73495024 | 1.0000000 |
| Degumille27-Bona15 | -2.059524e+00 | -4.49685500 | 0.37780738 | 0.2247383 |
| Degumille8-Bona15 | -3.541667e-01 | -2.72014582 | 2.01181249 | 1.0000000 |
| Degumille9-Bona15 | 8.833333e-01 | -1.76945886 | 3.53612552 | 0.9998744 |
| Degumille97-Bona15 | 8.333333e-01 | -1.99455264 | 3.66121931 | 0.9999842 |
| Bona7-Bona5 | 9.642857e-01 | -1.47304547 | 3.40161690 | 0.9982548 |
| Degumille27-Bona5 | -1.392857e+00 | -3.83018833 | 1.04447405 | 0.8876776 |
| Degumille8-Bona5 | 3.125000e-01 | -2.05347916 | 2.67847916 | 1.0000000 |
| Degumille9-Bona5 | 1.550000e+00 | -1.10279219 | 4.20279219 | 0.8656988 |
| Degumille97-Bona5 | 1.500000e+00 | -1.32788598 | 4.32788598 | 0.9421479 |
| Degumille27-Bona7 | -2.357143e+00 | -4.69885492 | -0.01543080 | 0.0464782* |
| Degumille8-Bona7 | -6.517857e-01 | -2.91913867 | 1.61556724 | 0.9999898 |
| Degumille9-Bona7 | 5.857143e-01 | -1.97950275 | 3.15093132 | 0.9999999 |
| Degumille97-Bona7 | 5.357143e-01 | -2.21018650 | 3.28161507 | 1.0000000 |
| Degumille8-Degumille27 | 1.705357e+00 | -0.56199581 | 3.97271010 | 0.4380630 |
| Degumille9-Degumille27 | 2.942857e+00 | 0.37764010 | 5.50807418 | 0.0084583** |
| Degumille97-Degumille27 | 2.892857e+00 | 0.14695635 | 5.63875793 | 0.0271157* |
| Degumille9-Degumille8 | 1.237500e+00 | -1.26002127 | 3.73502127 | 0.9710001 |
| Degumille97-Degumille8 | 1.187500e+00 | -1.49526819 | 3.87026819 | 0.9921868 |

Mean esterase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD1.

**Table C.11: Esterase activity of pseudo-papillae compared between genotypes in stage FD2 (*ES0*)**

| Comparison FD2 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 9.166667e-01 | -1.61267144 | 3.44600478 | 0.9995208 |
| Bona5-Bona12 | 3.333333e-01 | -2.76446054 | 3.43112721 | 1.0000000 |
| Bona7-Bona12 | 8.333333e-01 | -2.26446054 | 3.93112721 | 0.9999969 |
| Degumille27-Bona12 | 1.600000e+00 | -1.05279219 | 4.25279219 | 0.8291724 |
| Degumille8-Bona12 | 1.666667e+00 | -1.43112721 | 4.76446054 | 0.9338589 |
| Degumille9-Bona12 | 3.750000e-01 | -2.45288598 | 3.20288598 | 1.0000000 |
| Degumille97-Bona12 | 1.250000e-01 | -2.70288598 | 2.95288598 | 1.0000000 |
| Bona5-Bona15 | -5.833333e-01 | -3.68112721 | 2.51446054 | 1.0000000 |
| Bona7-Bona15 | -8.333333e-02 | -3.18112721 | 3.01446054 | 1.0000000 |
| Degumille27-Bona15 | 6.833333e-01 | -1.96945886 | 3.33612552 | 0.9999986 |
| Degumille8-Bona15 | 7.500000e-01 | -2.34779388 | 3.84779388 | 0.9999996 |
| Degumille9-Bona15 | -5.416667e-01 | -3.36955264 | 2.28621931 | 1.0000000 |
| Degumille97-Bona15 | -7.916667e-01 | -3.61955264 | 2.03621931 | 0.9999936 |
| Bona7-Bona5 | 5.000000e-01 | -3.07702426 | 4.07702426 | 1.0000000 |
| Degumille27-Bona5 | 1.266667e+00 | -1.93272109 | 4.46605443 | 0.9982375 |
| Degumille8-Bona5 | 1.333333e+00 | -2.24369093 | 4.91035759 | 0.9992668 |
| Degumille9-Bona5 | 4.166667e-02 | -3.30433314 | 3.38766648 | 1.0000000 |
| Degumille97-Bona5 | -2.083333e-01 | -3.55433314 | 3.13766648 | 1.0000000 |
| Degumille27-Bona7 | 7.666667e-01 | -2.43272109 | 3.96605443 | 0.9999997 |
| Degumille8-Bona7 | 8.333333e-01 | -2.74369093 | 4.41035759 | 0.9999998 |
| Degumille9-Bona7 | -4.583333e-01 | -3.80433314 | 2.88766648 | 1.0000000 |
| Degumille97-Bona7 | -7.083333e-01 | -4.05433314 | 2.63766648 | 1.0000000 |
| Degumille8-Degumille27 | 6.666667e-02 | -3.13272109 | 3.26605443 | 1.0000000 |
| Degumille9-Degumille27 | -1.225000e+00 | -4.16382531 | 1.71382531 | 0.9964155 |
| Degumille97-Degumille27 | -1.475000e+00 | -4.41382531 | 1.46382531 | 0.9667659 |

Mean esterase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD2.

**Table C.12: Esterase activity of pseudo-papillae compared between genotypes in stage FD3 (*ES0*)**

| Comparison FD3 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 1.625000e+00 | -1.31382531 | 4.56382531 | 0.9152312 |
| Bona5-Bona12 | -2.500000e-01 | -3.34779388 | 2.84779388 | 1.0000000 |
| Bona7-Bona12 | -8.928571e-02 | -2.83518650 | 2.65661507 | 1.0000000 |
| Degumille27-Bona12 | 1.375000e+00 | -1.72279388 | 4.47279388 | 0.9919123 |
| Degumille8-Bona12 | -5.416667e-01 | -3.88766648 | 2.80433314 | 1.0000000 |
| Degumille9-Bona12 | 9.250000e-01 | -2.01382531 | 3.86382531 | 0.9999507 |
| Degumille97-Bona12 | -2.500000e-01 | -3.34779388 | 2.84779388 | 1.0000000 |
| Bona5-Bona15 | -1.875000e+00 | -4.81382531 | 1.06382531 | 0.7496389 |
| Bona7-Bona15 | -1.714286e+00 | -4.27950275 | 0.85093132 | 0.6708298 |
| Degumille27-Bona15 | -2.500000e-01 | -3.18882531 | 2.68882531 | 1.0000000 |
| Degumille8-Bona15 | -2.166667e+00 | -5.36605443 | 1.03272109 | 0.6464068 |
| Degumille9-Bona15 | -7.000000e-01 | -3.47075108 | 2.07075108 | 0.9999990 |
| Degumille97-Bona15 | -1.875000e+00 | -4.81382531 | 1.06382531 | 0.7496389 |
| Bona7-Bona5 | 1.607143e-01 | -2.58518650 | 2.90661507 | 1.0000000 |
| Degumille27-Bona5 | 1.625000e+00 | -1.47279388 | 4.72279388 | 0.9480326 |
| Degumille8-Bona5 | -2.916667e-01 | -3.63766648 | 3.05433314 | 1.0000000 |
| Degumille9-Bona5 | 1.175000e+00 | -1.76382531 | 4.11382531 | 0.9979759 |
| Degumille97-Bona5 | 1.332268e-15 | -3.09779388 | 3.09779388 | 1.0000000 |
| Degumille27-Bona7 | 1.464286e+00 | -1.28161507 | 4.21018650 | 0.9391452 |
| Degumille8-Bona7 | -4.523810e-01 | -3.47551822 | 2.57075632 | 1.0000000 |
| Degumille9-Bona7 | 1.014286e+00 | -1.55093132 | 3.57950275 | 0.9982693 |
| Degumille97-Bona7 | -1.607143e-01 | -2.90661507 | 2.58518650 | 1.0000000 |
| Degumille8-Degumille27 | -1.916667e+00 | -5.26266648 | 1.42933314 | 0.8854668 |
| Degumille9-Degumille27 | -4.500000e-01 | -3.38882531 | 2.48882531 | 1.0000000 |
| Degumille97-Degumille27 | -1.625000e+00 | -4.72279388 | 1.47279388 | 0.9480326 |

Mean esterase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD3.

**Table C.13: Esterase activity of filaments compared between genotypes in stage FD1 (*ES0*)**

| Comparison FD1 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 2.380952e-01 | -3.33090376 | 3.8070942 | 1.0000000 |
| Bona5-Bona12 | -9.523810e-02 | -3.66423710 | 3.4737609 | 1.0000000 |
| Bona7-Bona12 | 2.285714e+00 | -1.14326904 | 5.7146976 | 0.6754151 |
| Degumille27-Bona12 | 4.285714e-01 | -3.00041190 | 3.8575548 | 1.0000000 |
| Degumille8-Bona12 | 9.464286e-01 | -2.37367026 | 4.2665274 | 0.9999912 |
| Degumille9-Bona12 | 3.321429e+00 | -0.69941079 | 7.3422679 | 0.2615699 |
| Degumille97-Bona12 | 1.821429e+00 | -2.19941079 | 5.8422679 | 0.9896058 |
| Bona5-Bona15 | -3.333333e-01 | -4.03705863 | 3.3703920 | 1.0000000 |
| Bona7-Bona15 | 2.047619e+00 | -1.52137995 | 5.6166180 | 0.8839833 |
| Degumille27-Bona15 | 1.904762e-01 | -3.37852281 | 3.7594752 | 1.0000000 |
| Degumille8-Bona15 | 7.083333e-01 | -2.75618445 | 4.1728511 | 1.0000000 |
| Degumille9-Bona15 | 3.083333e+00 | -1.05755744 | 7.2242241 | 0.4583815 |
| Degumille97-Bona15 | 1.583333e+00 | -2.55755744 | 5.7242241 | 0.9989316 |
| Bona7-Bona5 | 2.380952e+00 | -1.18804662 | 5.9499514 | 0.6739720 |
| Degumille27-Bona5 | 5.238095e-01 | -3.04518948 | 4.0928085 | 1.0000000 |
| Degumille8-Bona5 | 1.041667e+00 | -2.42285112 | 4.5061844 | 0.9999775 |
| Degumille9-Bona5 | 3.416667e+00 | -0.72422410 | 7.5575574 | 0.2635138 |
| Degumille97-Bona5 | 1.916667e+00 | -2.22422410 | 6.0575574 | 0.9865415 |
| Degumille27-Bona7 | -1.857143e+00 | -5.28612618 | 1.5718405 | 0.9296696 |
| Degumille8-Bona7 | -1.339286e+00 | -4.65938454 | 1.9808131 | 0.9977090 |
| Degumille9-Bona7 | 1.035714e+00 | -2.98512507 | 5.0565536 | 0.9999986 |
| Degumille97-Bona7 | -4.642857e-01 | -4.48512507 | 3.5565536 | 1.0000000 |
| Degumille8-Degumille27 | 5.178571e-01 | -2.80224169 | 3.8379560 | 1.0000000 |
| Degumille9-Degumille27 | 2.892857e+00 | -1.12798222 | 6.9136965 | 0.5279877 |
| Degumille97-Degumille27 | 1.392857e+00 | -2.62798222 | 5.4136965 | 0.9997629 |
| Degumille9-Degumille8 | 2.375000e+00 | -1.55339391 | 6.3033939 | 0.8261938 |
| Degumille97-Degumille8 | 8.750000e-01 | -3.05339391 | 4.8033939 | 0.9999999 |

Mean esterase activity of filaments (fi) compared between the individual genotypes in stage FD1.

**Table C.14: Esterase activity of filaments compared between genotypes in stage FD2 (*ES0*)**

| Comparison FD2 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 1.166667e+00 | -2.53705863 | 4.8703920 | 0.9999500 |
| Bona5-Bona12 | -3.333333e-01 | -4.86945190 | 4.2027852 | 1.0000000 |
| Bona7-Bona12 | 6.666667e-01 | -3.86945190 | 5.2027852 | 1.0000000 |
| Degumille27-Bona12 | 2.933333e+00 | -0.95116653 | 6.8178332 | 0.4300507 |
| Degumille8-Bona12 | 8.881784e-16 | -4.53611857 | 4.5361186 | 1.0000000 |
| Degumille9-Bona12 | 3.333333e+00 | -0.80755744 | 7.4742241 | 0.3068679 |
| Degumille97-Bona12 | 8.333333e-01 | -3.30755744 | 4.9742241 | 1.0000000 |
| Bona5-Bona15 | -1.500000e+00 | -6.03611857 | 3.0361186 | 0.9998879 |
| Bona7-Bona15 | -5.000000e-01 | -5.03611857 | 4.0361186 | 1.0000000 |
| Degumille27-Bona15 | 1.766667e+00 | -2.11783320 | 5.6511665 | 0.9890931 |
| Degumille8-Bona15 | -1.166667e+00 | -5.70278523 | 3.3694519 | 0.9999987 |
| Degumille9-Bona15 | 2.166667e+00 | -1.97422410 | 6.3075574 | 0.9493051 |
| Degumille97-Bona15 | -3.333333e-01 | -4.47422410 | 3.8075574 | 1.0000000 |
| Bona7-Bona5 | 1.000000e+00 | -4.23785855 | 6.2378586 | 1.0000000 |
| Degumille27-Bona5 | 3.266667e+00 | -1.41821644 | 7.9515498 | 0.5904446 |
| Degumille8-Bona5 | 3.333333e-01 | -4.90452522 | 5.5711919 | 1.0000000 |
| Degumille9-Bona5 | 3.666667e+00 | -1.23290137 | 8.5662347 | 0.4481993 |
| Degumille97-Bona5 | 1.166667e+00 | -3.73290137 | 6.0662347 | 0.9999997 |
| Degumille27-Bona7 | 2.266667e+00 | -2.41821644 | 6.9515498 | 0.9776369 |
| Degumille8-Bona7 | -6.666667e-01 | -5.90452522 | 4.5711919 | 1.0000000 |
| Degumille9-Bona7 | 2.666667e+00 | -2.23290137 | 7.5662347 | 0.9264405 |
| Degumille97-Bona7 | 1.666667e-01 | -4.73290137 | 5.0662347 | 1.0000000 |
| Degumille8-Degumille27 | -2.933333e+00 | -7.61821644 | 1.7515498 | 0.7783188 |
| Degumille9-Degumille27 | 4.000000e-01 | -3.90333992 | 4.7033399 | 1.0000000 |
| Degumille97-Degumille27 | -2.100000e+00 | -6.40333992 | 2.2033399 | 0.9754172 |

Mean esterase activity of filaments (fi) compared between the individual genotypes in stage FD2.

**Table C.15: Esterase activity of filaments compared between genotypes in stage FD3 (*ES0*)**

| Comparison FD3 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 8.000000e-01 | -3.25722779 | 4.8572278 | 1.0000000 |
| Bona5-Bona12 | -9.000000e-01 | -5.20333992 | 3.4033399 | 1.0000000 |
| Bona7-Bona12 | -4.000000e-01 | -4.15626303 | 3.3562630 | 1.0000000 |
| Degumille27-Bona12 | 1.350000e+00 | -2.95333992 | 5.6533399 | 0.9999534 |
| Degumille8-Bona12 | 2.666667e-01 | -4.41821644 | 4.9515498 | 1.0000000 |
| Degumille9-Bona12 | 4.000000e-01 | -3.65722779 | 4.4572278 | 1.0000000 |
| Degumille97-Bona12 | 1.600000e+00 | -2.70333992 | 5.9033399 | 0.9992941 |
| Bona5-Bona15 | -1.700000e+00 | -6.00333992 | 2.6033399 | 0.9982913 |
| Bona7-Bona15 | -1.200000e+00 | -4.95626303 | 2.5562630 | 0.9999367 |
| Degumille27-Bona15 | 5.500000e-01 | -3.75333992 | 4.8533399 | 1.0000000 |
| Degumille8-Bona15 | -5.333333e-01 | -5.21821644 | 4.1515498 | 1.0000000 |
| Degumille9-Bona15 | -4.000000e-01 | -4.45722779 | 3.6572278 | 1.0000000 |
| Degumille97-Bona15 | 8.000000e-01 | -3.50333992 | 5.1033399 | 1.0000000 |
| Bona7-Bona5 | 5.000000e-01 | -3.52083936 | 4.5208394 | 1.0000000 |
| Degumille27-Bona5 | 2.250000e+00 | -2.28611857 | 6.7861186 | 0.9706680 |
| Degumille8-Bona5 | 1.166667e+00 | -3.73290137 | 6.0662347 | 0.9999997 |
| Degumille9-Bona5 | 1.300000e+00 | -3.00333992 | 5.6033399 | 0.9999756 |
| Degumille97-Bona5 | 2.500000e+00 | -2.03611857 | 7.0361186 | 0.9176579 |
| Degumille27-Bona7 | 1.750000e+00 | -2.27083936 | 5.7708394 | 0.9936885 |
| Degumille8-Bona7 | 6.666667e-01 | -3.76013177 | 5.0934651 | 1.0000000 |
| Degumille9-Bona7 | 8.000000e-01 | -2.95626303 | 4.5562630 | 1.0000000 |
| Degumille97-Bona7 | 2.000000e+00 | -2.02083936 | 6.0208394 | 0.9697792 |
| Degumille8-Degumille27 | -1.083333e+00 | -5.98290137 | 3.8162347 | 0.9999999 |
| Degumille9-Degumille27 | -9.500000e-01 | -5.25333992 | 3.3533399 | 0.9999999 |
| Degumille97-Degumille27 | 2.500000e-01 | -4.28611857 | 4.7861186 | 1.0000000 |

Mean esterase activity of filaments (fi) compared between the individual genotypes in stage FD3.

# Appendix C.2: Post-hoc comparisons of test series *ES1*

**Table C.16: Comparison between the individual stages of floral development (*ES1*)**

| **Papillae** | | | | |
|---|---|---|---|---|
| Comparison | diff | lwr | upr | p adj |
| FD2-FD1 | - 0.3917173 | - 1.1138170 | 0.33038239 | 0.4063865 |
| FD3-FD1 | - 0.6403437 | - 1.3750073 | 0.09431986 | 0.1010689 |
| FD3-FD2 | - 0.2486264 | - 0.9515978 | 0.45434509 | 0.6805803 |
| **Pseudo-Papillae** | | | | |
| Comparison | diff | lwr | upr | p adj |
| FD2-FD1 | - 1.4567003 | - 2.0749725 | - 0.8384281 | 0.0000003*** |
| FD3-FD1 | - 1.8587470 | - 2.4847326 | - 1.2327615 | 0.0000000*** |
| FD3-FD2 | - 0.4020468 | - 0.9979512 | 0.1938576 | 0.2501583 |
| **Filament** | | | | |
| Comparison | diff | lwr | upr | p adj |
| FD2-FD1 | 1.1243001 | 0.20707881 | 2.041521 | 0.0118048* |
| FD3-FD1 | 1.9335106 | 1.01259576 | 2.854426 | 0.0000052*** |
| FD3-FD2 | 0.8092105 | - 0.06668357 | 1.685105 | 0.0767153 |

Mean esterase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) compared between the individual stages of floral development (FD1, FD2, FD3).

**Table C.17: Comparison between self-compatible and self-incompatible genotypes (*ES1*)**

| SI-SC | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| diff | - 0.58458210 | - 0.3445513 | - 0.1241401 |
| lwr | - 1.07039900 | - 0.8236750 | - 0.7829938 |
| upr | - 0.09876479 | 0.1345725 | 0.5347136 |
| p adj | 0.0186796* | 0.1574623 | 0.7102847 |

Mean esterase activity compared between self-compatible (SC) and self-incompatible (SI) genotypes.

**Table C.18: Comparison between SC and SI genotypes at different stages of floral development (*ES1*)**

| **Papillae** | | | | |
|---|---|---|---|---|
| SI-SC | diff | lwr | upr | p adj |
| FD1 | 0.89818182 | - 0.2812578 | 2.07762140 | 0.2445974 |
| FD2 | - 2.07354839 | - 3.1581026 | - 0.98899415 | 0.0000022*** |
| FD3 | - 0.35119048 | - 1.4735323 | 0.77115135 | 0.9451058 |
| **Pseudo-Papillae** | | | | |
| SI-SC | diff | lwr | upr | p adj |
| FD1 | 1.04000000 | 0.007161196 | 2.07283880 | 0.0473617* |
| FD2 | - 1.38312500 | - 2.326224243 | - 0.44002576 | 0.0005598*** |
| FD3 | - 0.62637363 | - 1.588641866 | 0.33589461 | 0.4191418 |
| **Filaments** | | | | |
| SI-SC | diff | lwr | upr | p adj |
| FD1 | - 0.4945455 | - 2.16159408 | 1.1725032 | 0.9561366 |
| FD2 | 0.4350000 | - 1.08720491 | 1.9572049 | 0.9625612 |
| FD3 | - 0.2500000 | - 1.77411124 | 1.2741112 | 0.9969980 |

Mean esterase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) compared between self-compatible (SC) and self-incompatible (SI) genotypes at different floral development stages (FD1, FD2, FD3).

**Table C.19: Mean esterase activity of papillae compared between genotypes (*ES1*)**

| Comparison (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 1.13822906 | -0.1714918 | 2.44794995 | 0.1390322 |
| Bona22-Bona12 | 0.78851497 | -0.3779343 | 1.95496425 | 0.4317262 |
| Bona5-Bona12 | 0.52272727 | -0.6566113 | 1.70206589 | 0.8711233 |
| Bona7-Bona12 | 0.83249035 | -0.4106417 | 2.07562241 | 0.4443524 |
| Degumille27-Bona12 | 1.39855928 | 0.1354778 | 2.66164075 | 0.0189486* |
| Degumille44-Bona12 | 0.05715065 | -1.1678566 | 1.28215787 | 0.9999999 |
| Degumille9-Bona12 | 0.50451907 | -0.7204882 | 1.72952629 | 0.9086824 |
| Bona22-Bona19 | -0.34971409 | -1.6478409 | 0.94841270 | 0.9910910 |
| Bona5-Bona19 | -0.61550179 | -1.9252227 | 0.69421911 | 0.8330970 |
| Bona7-Bona19 | -0.30573871 | -1.6731840 | 1.06170656 | 0.9971730 |
| Degumille27-Bona19 | 0.26033022 | -1.1252758 | 1.64593624 | 0.9990723 |
| Degumille44-Bona19 | -1.08107841 | -2.4320677 | 0.26991083 | 0.2199076 |
| Degumille9-Bona19 | -0.63370999 | -1.9846992 | 0.71727925 | 0.8344318 |
| Bona5-Bona22 | -0.26578769 | -1.4322370 | 0.90066159 | 0.9968155 |
| Bona7-Bona22 | 0.04397539 | -1.1869355 | 1.27488630 | 1.0000000 |
| Degumille27-Bona22 | 0.61004431 | -0.6410109 | 1.86109954 | 0.8050516 |
| Degumille44-Bona22 | -0.73136432 | -1.9439677 | 0.48123910 | 0.5820022 |
| Degumille9-Bona22 | -0.28399590 | -1.4965993 | 0.92860752 | 0.9962189 |
| Bona7-Bona5 | 0.30976308 | -0.9333690 | 1.55289514 | 0.9944519 |
| Degumille27-Bona5 | 0.87583201 | -0.3872495 | 2.13891348 | 0.3976613 |
| Degumille44-Bona5 | -0.46557663 | -1.6905839 | 0.75943060 | 0.9387830 |
| Degumille9-Bona5 | -0.01820821 | -1.2432154 | 1.20679902 | 1.0000000 |
| Degumille27-Bona7 | 0.56606893 | -0.7567737 | 1.88891153 | 0.8904331 |
| Degumille44-Bona7 | -0.77533971 | -2.0618779 | 0.51119845 | 0.5830061 |
| Degumille9-Bona7 | -0.32797129 | -1.6145094 | 0.95856687 | 0.9936214 |
| Degumille44-Degumille27 | -1.34140863 | -2.6472333 | -0.03558401 | 0.0394962* |
| Degumille9-Degumille27 | -0.89404021 | -2.1998648 | 0.41178441 | 0.4146494 |
| Degumille9-Degumille44 | 0.44736842 | -0.8216650 | 1.71640184 | 0.9587172 |

Mean esterase activity of papillae (p) compared between the individual genotypes.

**Table C.20: Esterase activity of pseudo-papillae compared between genotypes (*ES1*)**

| Comparison (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 0.37168048 | -0.7292229 | 1.4725839 | 0.9674236 |
| Bona22-Bona12 | 0.48433861 | -0.5148782 | 1.4835555 | 0.8101339 |
| Bona5-Bona12 | 0.07728425 | -0.9117030 | 1.0662715 | 0.9999975 |
| Bona7-Bona12 | 0.81928090 | -0.2456248 | 1.8841866 | 0.2652264 |
| Degumille27-Bona12 | 1.32796012 | 0.2459651 | 2.4099551 | 0.0056275** |
| Degumille44-Bona12 | -0.16444786 | -1.2138273 | 0.8849315 | 0.9997198 |
| Degumille9-Bona12 | -0.11181628 | -1.1611957 | 0.9375631 | 0.9999793 |
| Bona22-Bona19 | 0.11265813 | -0.9781218 | 1.2034381 | 0.9999833 |
| Bona5-Bona19 | -0.29439623 | -1.3758131 | 0.7870206 | 0.9905442 |
| Bona7-Bona19 | 0.44760042 | -0.7036557 | 1.5988566 | 0.9314554 |
| Degumille27-Bona19 | 0.95627964 | -0.2108021 | 2.1233614 | 0.1948544 |
| Degumille44-Bona19 | -0.53612834 | -1.6730381 | 0.6007814 | 0.8309245 |
| Degumille9-Bona19 | -0.48349676 | -1.6204065 | 0.6534130 | 0.8938019 |
| Bona5-Bona22 | -0.40705436 | -1.3847600 | 0.5706513 | 0.9040011 |
| Bona7-Bona22 | 0.33494229 | -0.7194944 | 1.3893790 | 0.9767916 |
| Degumille27-Bona22 | 0.84362151 | -0.2280714 | 1.9153144 | 0.2384776 |
| Degumille44-Bona22 | -0.64878648 | -1.6875404 | 0.3899674 | 0.5375110 |
| Degumille9-Bona22 | -0.59615490 | -1.6349088 | 0.4425990 | 0.6430812 |
| Bona7-Bona5 | 0.74199665 | -0.3027513 | 1.7867446 | 0.3662327 |
| Degumille27-Bona5 | 1.25067587 | 0.1885143 | 2.3128375 | 0.0094534** |
| Degumille44-Bona5 | -0.24173211 | -1.2706496 | 0.7871854 | 0.9961571 |
| Degumille9-Bona5 | -0.18910053 | -1.2180180 | 0.8398170 | 0.9991991 |
| Degumille27-Bona7 | 0.50867922 | -0.6245090 | 1.6418675 | 0.8637929 |
| Degumille44-Bona7 | -0.98372876 | -2.0858175 | 0.1183600 | 0.1174005 |
| Degumille9-Bona7 | -0.93109718 | -2.0331859 | 0.1709915 | 0.1645652 |
| Degumille44-Degumille27 | -1.49240798 | -2.6110181 | -0.3737979 | 0.0017309** |
| Degumille9-Degumille27 | -1.43977641 | -2.5583865 | -0.3211663 | 0.0029411** |
| Degumille9-Degumille44 | 0.05263158 | -1.0344620 | 1.1397252 | 0.9999999 |

Mean esterase activity of pseudo-papillae (fp) compared between the individual genotypes.

**Table C.21: Esterase activity of filaments compared between genotypes (*ES1*)**

| Comparison (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 1.19626698 | -0.52852834 | 2.9210623 | 0.3979265 |
| Bona22-Bona12 | 2.36785450 | 0.74945342 | 3.9862556 | 0.0003712*** |
| Bona5-Bona12 | -0.18294596 | -1.78477851 | 1.4188866 | 0.9999668 |
| Bona7-Bona12 | 1.64761699 | -0.07717833 | 3.3724123 | 0.0724654 |
| Degumille27-Bona12 | 0.91957426 | -0.83290007 | 2.6720486 | 0.7402413 |
| Degumille44-Bona12 | 0.81879732 | -0.88085053 | 2.5184452 | 0.8151438 |
| Degumille9-Bona12 | 1.66090258 | -0.03874527 | 3.3605504 | 0.0605653 |
| Bona22-Bona19 | 1.17158752 | -0.53625145 | 2.8794265 | 0.4126012 |
| Bona5-Bona19 | -1.37921294 | -3.07135933 | 0.3129335 | 0.2004575 |
| Bona7-Bona19 | 0.45135001 | -1.35763058 | 2.2603306 | 0.9944368 |
| Degumille27-Bona19 | -0.27669272 | -2.11208318 | 1.5586977 | 0.9997840 |
| Degumille44-Bona19 | -0.37746966 | -2.16248919 | 1.4075499 | 0.9980250 |
| Degumille9-Bona19 | 0.46463560 | -1.32038392 | 2.2496551 | 0.9927965 |
| Bona5-Bona22 | -2.55080046 | -4.13436056 | -0.9672404 | 0.0000559*** |
| Bona7-Bona22 | -0.72023752 | -2.42807649 | 0.9876015 | 0.8980665 |
| Degumille27-Bona22 | -1.44828024 | -3.18406863 | 0.2875081 | 0.1766779 |
| Degumille44-Bona22 | -1.54905719 | -3.23149526 | 0.1333809 | 0.0950793 |
| Degumille9-Bona22 | -0.70695192 | -2.38939000 | 0.9754862 | 0.8998124 |
| Bona7-Bona5 | 1.83056294 | 0.13841655 | 3.5227093 | 0.0240655* |
| Degumille27-Bona5 | 1.10252021 | -0.61783056 | 2.8228710 | 0.5038397 |
| Degumille44-Bona5 | 1.00174327 | -0.66476306 | 2.6682496 | 0.5869366 |
| Degumille9-Bona5 | 1.84384854 | 0.17734221 | 3.5103549 | 0.0190683* |
| Degumille27-Bona7 | -0.72804273 | -2.56343319 | 1.1073477 | 0.9243237 |
| Degumille44-Bona7 | -0.82881967 | -2.61383919 | 0.9561999 | 0.8420346 |
| Degumille9-Bona7 | 0.01328559 | -1.77173393 | 1.7983051 | 1.0000000 |
| Degumille44-Degumille27 | -0.10077694 | -1.91255565 | 1.7110018 | 0.9999998 |
| Degumille9-Degumille27 | 0.74132832 | -1.07045038 | 2.5531070 | 0.9118434 |
| Degumille9-Degumille44 | 0.84210526 | -0.91862715 | 2.6028377 | 0.8206805 |

Mean esterase activity of filaments (fi) compared between the individual genotypes.

**Table C.22: Esterase activity of papillae compared between genotypes in stage FD1 (*ES1*)**

| Comparison FD1 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 3.857143e-01 | -2.37736084 | 3.148789414 | 1.0000000 |
| Bona22-Bona12 | 8.857143e-01 | -1.87736084 | 3.648789414 | 0.9999496 |
| Bona5-Bona12 | -1.714286e+00 | -4.23661667 | 0.808045245 | 0.6546310 |
| Bona7-Bona12 | 8.690476e-01 | -1.75627768 | 3.494372918 | 0.9999134 |
| Degumille27-Bona12 | -1.214286e+00 | -3.97736084 | 1.548789414 | 0.9940428 |
| Degumille44-Bona12 | -1.309524e-01 | -2.75627768 | 2.494372918 | 1.0000000 |
| Degumille9-Bona12 | 7.857143e-01 | -1.83961101 | 3.411039585 | 0.9999847 |
| Bona22-Bona19 | 5.000000e-01 | -2.48446224 | 3.484462239 | 1.0000000 |
| Bona5-Bona19 | -2.100000e+00 | -4.86307513 | 0.663075128 | 0.4290494 |
| Bona7-Bona19 | 4.833333e-01 | -2.37407172 | 3.340738383 | 1.0000000 |
| Degumille27-Bona19 | -1.600000e+00 | -4.58446224 | 1.384462239 | 0.9427501 |
| Degumille44-Bona19 | -5.166667e-01 | -3.37407172 | 2.340738383 | 1.0000000 |
| Degumille9-Bona19 | 4.000000e-01 | -2.45740505 | 3.257405050 | 1.0000000 |
| Bona5-Bona22 | -2.600000e+00 | -5.36307513 | 0.163075128 | 0.0945105 |
| Bona7-Bona22 | -1.666667e-02 | -2.87407172 | 2.840738383 | 1.0000000 |
| Degumille27-Bona22 | -2.100000e+00 | -5.08446224 | 0.884462239 | 0.5871118 |
| Degumille44-Bona22 | -1.016667e+00 | -3.87407172 | 1.840738383 | 0.9997198 |
| Degumille9-Bona22 | -1.000000e-01 | -2.95740505 | 2.757405050 | 1.0000000 |
| Bona7-Bona5 | 2.583333e+00 | -0.04199197 | 5.208658632 | 0.0597779 |
| Degumille27-Bona5 | 5.000000e-01 | -2.26307513 | 3.263075128 | 1.0000000 |
| Degumille44-Bona5 | 1.583333e+00 | -1.04199197 | 4.208658632 | 0.8414081 |
| Degumille9-Bona5 | 2.500000e+00 | -0.12532530 | 5.125325299 | 0.0840859 |
| Degumille27-Bona7 | -2.083333e+00 | -4.94073838 | 0.774071717 | 0.5147174 |
| Degumille44-Bona7 | -1.000000e+00 | -3.72442882 | 1.724428817 | 0.9995438 |
| Degumille9-Bona7 | -8.333333e-02 | -2.80776215 | 2.641095484 | 1.0000000 |
| Degumille44-Degumille27 | 1.083333e+00 | -1.77407172 | 3.940738383 | 0.9992520 |
| Degumille9-Degumille27 | 2.000000e+00 | -0.85740505 | 4.857405050 | 0.5976473 |

Mean esterase activity of papillae (p) compared between the individual genotypes in stage FD1.

**Table C.23: Esterase activity of papillae compared between genotypes in stage FD2 (*ES1*)**

| Comparison FD2 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 1.785714e+00 | -0.73661667 | 4.308045245 | 0.5748243 |
| Bona22-Bona12 | 6.142857e-01 | -1.71118853 | 2.939759956 | 0.9999984 |
| Bona5-Bona12 | 2.785714e+00 | 0.26338333 | 5.308045245 | 0.0140930* |
| Bona7-Bona12 | 4.761905e-02 | -2.57770625 | 2.672944347 | 1.0000000 |
| Degumille27-Bona12 | 3.000000e+00 | 0.47766904 | 5.522330960 | 0.0044893** |
| Degumille44-Bona12 | -7.857143e-01 | -3.41103958 | 1.839611013 | 0.9999847 |
| Degumille9-Bona12 | 4.761905e-02 | -2.57770625 | 2.672944347 | 1.0000000 |
| Bona22-Bona19 | -1.171429e+00 | -3.49690281 | 1.154045670 | 0.9697577 |
| Bona5-Bona19 | 1.000000e+00 | -1.52233096 | 3.522330960 | 0.9985529 |
| Bona7-Bona19 | -1.738095e+00 | -4.36342054 | 0.887230061 | 0.7024191 |
| Degumille27-Bona19 | 1.214286e+00 | -1.30804525 | 3.736616674 | 0.9817667 |
| Degumille44-Bona19 | -2.571429e+00 | -5.19675387 | 0.053896728 | 0.0628325 |
| Degumille9-Bona19 | -1.738095e+00 | -4.36342054 | 0.887230061 | 0.7024191 |
| Bona5-Bona22 | 2.171429e+00 | -0.15404567 | 4.496902813 | 0.1017262 |
| Bona7-Bona22 | -5.666667e-01 | -3.00346988 | 1.870136548 | 0.9999999 |
| Degumille27-Bona22 | 2.385714e+00 | 0.06024004 | 4.711188528 | 0.0371023* |
| Degumille44-Bona22 | -1.400000e+00 | -3.83680321 | 1.036803214 | 0.8926109 |
| Degumille9-Bona22 | -5.666667e-01 | -3.00346988 | 1.870136548 | 0.9999999 |
| Bona7-Bona5 | -2.738095e+00 | -5.36342054 | -0.112769939 | 0.0303078* |
| Degumille27-Bona5 | 2.142857e-01 | -2.30804525 | 2.736616674 | 1.0000000 |
| Degumille44-Bona5 | -3.571429e+00 | -6.19675387 | -0.946103272 | 0.0003485*** |
| Degumille9-Bona5 | -2.738095e+00 | -5.36342054 | -0.112769939 | 0.0303078* |
| Degumille27-Bona7 | 2.952381e+00 | 0.32705565 | 5.577706251 | 0.0108355* |
| Degumille44-Bona7 | -8.333333e-01 | -3.55776215 | 1.891095484 | 0.9999775 |
| Degumille9-Bona7 | -2.664535e-15 | -2.72442882 | 2.724428817 | 1.0000000 |

Mean esterase activity of papillae (p) compared between the individual genotypes in stage FD2.

**Table C.24: Esterase activity of papillae compared between genotypes in stage FD3 (*ES1*)**

| Comparison FD3 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 8.750000e-01 | -2.31967597 | 4.069675965 | 0.9999969 |
| Bona22-Bona12 | 1.250000e+00 | -1.10942457 | 3.609424567 | 0.9490363 |
| Bona5-Bona12 | 5.000000e-01 | -1.85942457 | 2.859424567 | 1.0000000 |
| Bona7-Bona12 | 1.541667e+00 | -1.00680314 | 4.090136469 | 0.8377146 |
| Degumille27-Bona12 | 1.875000e+00 | -0.81515791 | 4.565157908 | 0.6060116 |
| Degumille44-Bona12 | 9.464286e-01 | -1.49580788 | 3.388665021 | 0.9989630 |
| Degumille9-Bona12 | 6.607143e-01 | -1.78152216 | 3.102950736 | 0.9999975 |
| Bona22-Bona19 | 3.750000e-01 | -2.81967597 | 3.569675965 | 1.0000000 |
| Bona5-Bona19 | -3.750000e-01 | -3.56967597 | 2.819675965 | 1.0000000 |
| Bona7-Bona19 | 6.666667e-01 | -2.67006355 | 4.003396888 | 1.0000000 |
| Degumille27-Bona19 | 1.000000e+00 | -2.44616015 | 4.446160154 | 0.9999912 |
| Degumille44-Bona19 | 7.142857e-02 | -3.18488670 | 3.327743838 | 1.0000000 |
| Degumille9-Bona19 | -2.142857e-01 | -3.47060098 | 3.042029552 | 1.0000000 |
| Bona5-Bona22 | -7.500000e-01 | -3.10942457 | 1.609424567 | 0.9999563 |
| Bona7-Bona22 | 2.916667e-01 | -2.25680314 | 2.840136469 | 1.0000000 |
| Degumille27-Bona22 | 6.250000e-01 | -2.06515791 | 3.315157908 | 0.9999999 |
| Degumille44-Bona22 | -3.035714e-01 | -2.74580788 | 2.138665021 | 1.0000000 |
| Degumille9-Bona22 | -5.892857e-01 | -3.03152216 | 1.852950736 | 0.9999997 |
| Bona7-Bona5 | 1.041667e+00 | -1.50680314 | 3.590136469 | 0.9977643 |
| Degumille27-Bona5 | 1.375000e+00 | -1.31515791 | 4.065157908 | 0.9646833 |
| Degumille44-Bona5 | 4.464286e-01 | -1.99580788 | 2.888665021 | 1.0000000 |
| Degumille9-Bona5 | 1.607143e-01 | -2.28152216 | 2.602950736 | 1.0000000 |
| Degumille27-Bona7 | 3.333333e-01 | -2.52407172 | 3.190738383 | 1.0000000 |
| Degumille44-Bona7 | -5.952381e-01 | -3.22056339 | 2.030087204 | 0.9999999 |
| Degumille9-Bona7 | -8.809524e-01 | -3.50627768 | 1.744372918 | 0.9998914 |

Mean esterase activity of papillae (p) compared between the individual genotypes in stage FD3.

**Table C.25: Esterase activity of pseudo-papillae compared between genotypes in stage FD1 (*ES1*)**

| Comparison FD1 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | -7.000000e-01 | -3.06666490 | 1.666664904 | 0.9999878 |
| Bona22-Bona12 | -7.000000e-01 | -3.06666490 | 1.666664904 | 0.9999878 |
| Bona5-Bona12 | -1.071429e+00 | -3.23188816 | 1.089031019 | 0.9747223 |
| Bona7-Bona12 | 7.500000e-01 | -1.49867764 | 2.998677636 | 0.9999035 |
| Degumille27-Bona12 | -1.500000e+00 | -3.86666490 | 0.866664904 | 0.7747597 |
| Degumille44-Bona12 | -2.500000e-01 | -2.49867764 | 1.998677636 | 1.0000000 |
| Degumille9-Bona12 | -3.333333e-01 | -2.58201097 | 1.915344303 | 1.0000000 |
| Bona22-Bona19 | -1.776357e-15 | -2.55629026 | 2.556290261 | 1.0000000 |
| Bona5-Bona19 | -3.714286e-01 | -2.73809348 | 1.995236333 | 1.0000000 |
| Bona7-Bona19 | 1.450000e+00 | -0.99746159 | 3.897461591 | 0.8625623 |
| Degumille27-Bona19 | -8.000000e-01 | -3.35629026 | 1.756290261 | 0.9999672 |
| Degumille44-Bona19 | 4.500000e-01 | -1.99746159 | 2.897461591 | 1.0000000 |
| Degumille9-Bona19 | 3.666667e-01 | -2.08079492 | 2.814128258 | 1.0000000 |
| Bona5-Bona22 | -3.714286e-01 | -2.73809348 | 1.995236333 | 1.0000000 |
| Bona7-Bona22 | 1.450000e+00 | -0.99746159 | 3.897461591 | 0.8625623 |
| Degumille27-Bona22 | -8.000000e-01 | -3.35629026 | 1.756290261 | 0.9999672 |
| Degumille44-Bona22 | 4.500000e-01 | -1.99746159 | 2.897461591 | 1.0000000 |
| Degumille9-Bona22 | 3.666667e-01 | -2.08079492 | 2.814128258 | 1.0000000 |
| Bona7-Bona5 | 1.821429e+00 | -0.42724906 | 4.070106207 | 0.3046305 |
| Degumille27-Bona5 | -4.285714e-01 | -2.79523633 | 1.938093476 | 1.0000000 |
| Degumille44-Bona5 | 8.214286e-01 | -1.42724906 | 3.070106207 | 0.9995829 |
| Degumille9-Bona5 | 7.380952e-01 | -1.51058240 | 2.986772874 | 0.9999261 |
| Degumille27-Bona7 | -2.250000e+00 | -4.69746159 | 0.197461591 | 0.1176189 |
| Degumille44-Bona7 | -1.000000e+00 | -3.33356307 | 1.333563065 | 0.9957771 |
| Degumille9-Bona7 | -1.083333e+00 | -3.41689640 | 1.250229732 | 0.9882590 |
| Degumille44-Degumille27 | 1.250000e+00 | -1.19746159 | 3.697461591 | 0.9652484 |
| Degumille9-Degumille27 | 1.166667e+00 | -1.28079492 | 3.614128258 | 0.9838989 |

Mean esterase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD1.

**Table C.26: Esterase activity of pseudo-papillae compared between genotypes in stage FD2 (*ES1*)**

| Comparison FD2 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 1.785714e+00 | -0.37474530 | 3.946173876 | 0.2682742 |
| Bona22-Bona12 | 7.571429e-01 | -1.23470247 | 2.748988181 | 0.9992334 |
| Bona5-Bona12 | 1.544643e+00 | -0.54721315 | 3.636498860 | 0.4893400 |
| Bona7-Bona12 | 8.571429e-01 | -1.39153478 | 3.105820493 | 0.9992007 |
| Degumille27-Bona12 | 2.500000e+00 | 0.33954041 | 4.660459590 | 0.0069777** |
| Degumille44-Bona12 | -5.595238e-01 | -2.80820145 | 1.689153826 | 0.9999995 |
| Degumille9-Bona12 | 4.404762e-01 | -1.80820145 | 2.689153826 | 1.0000000 |
| Bona22-Bona19 | -1.028571e+00 | -3.02041675 | 0.963273895 | 0.9609977 |
| Bona5-Bona19 | -2.410714e-01 | -2.33292743 | 1.850784575 | 1.0000000 |
| Bona7-Bona19 | -9.285714e-01 | -3.17724906 | 1.320106207 | 0.9974539 |
| Degumille27-Bona19 | 7.142857e-01 | -1.44617388 | 2.874745304 | 0.9999166 |
| Degumille44-Bona19 | -2.345238e+00 | -4.59391573 | -0.096560459 | 0.0302884* |
| Degumille9-Bona19 | -1.345238e+00 | -3.59391573 | 0.903439541 | 0.8516749 |
| Bona5-Bona22 | 7.875000e-01 | -1.12971770 | 2.704717695 | 0.9976360 |
| Bona7-Bona22 | 1.000000e-01 | -1.98720226 | 2.187202258 | 1.0000000 |
| Degumille27-Bona22 | 1.742857e+00 | -0.24898818 | 3.734702467 | 0.1784346 |
| Degumille44-Bona22 | -1.316667e+00 | -3.40386892 | 0.770535591 | 0.7818024 |
| Degumille9-Bona22 | -3.166667e-01 | -2.40386892 | 1.770535591 | 1.0000000 |
| Bona7-Bona5 | -6.875000e-01 | -2.87034837 | 1.495348370 | 0.9999634 |
| Degumille27-Bona5 | 9.553571e-01 | -1.13649886 | 3.047213146 | 0.9903971 |
| Degumille44-Bona5 | -2.104167e+00 | -4.28701504 | 0.078681704 | 0.0743524 |
| Degumille9-Bona5 | -1.104167e+00 | -3.28701504 | 1.078681704 | 0.9686367 |
| Degumille27-Bona7 | 1.642857e+00 | -0.60582049 | 3.891534779 | 0.5113622 |
| Degumille44-Bona7 | -1.416667e+00 | -3.75022973 | 0.916896399 | 0.8341066 |
| Degumille9-Bona7 | -4.166667e-01 | -2.75022973 | 1.916896399 | 1.0000000 |

Mean esterase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD2.

**Table C.27: Esterase activity of pseudo-papillae compared between genotypes in stage FD3 (*ES1*)**

| Comparison FD3 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | -5.000000e-01 | -2.97511740 | 1.975117402 | 1.0000000 |
| Bona22-Bona12 | 1.312500e+00 | -0.70842490 | 3.333424896 | 0.7361239 |
| Bona5-Bona12 | -2.500000e-01 | -2.21398598 | 1.713985975 | 1.0000000 |
| Bona7-Bona12 | 8.333333e-01 | -1.34951504 | 3.016181704 | 0.9991820 |
| Degumille27-Bona12 | 2.750000e+00 | 0.44579110 | 5.054208902 | 0.0042183** |
| Degumille44-Bona12 | 2.500000e-01 | -1.84185600 | 2.341856003 | 1.0000000 |
| Degumille9-Bona12 | -3.928571e-01 | -2.48471315 | 1.698998860 | 1.0000000 |
| Bona22-Bona19 | 1.812500e+00 | -0.66261740 | 4.287617402 | 0.5065578 |
| Bona5-Bona19 | 2.500000e-01 | -2.17884945 | 2.678849446 | 1.0000000 |
| Bona7-Bona19 | 1.333333e+00 | -1.27566949 | 3.942336155 | 0.9650193 |
| Degumille27-Bona19 | 3.250000e+00 | 0.53864473 | 5.961355267 | 0.0039200** |
| Degumille44-Bona19 | 7.500000e-01 | -1.78336343 | 3.283363427 | 0.9999876 |
| Degumille9-Bona19 | 1.071429e-01 | -2.42622057 | 2.640506285 | 1.0000000 |
| Bona5-Bona22 | -1.562500e+00 | -3.52648598 | 0.401485975 | 0.3385435 |
| Bona7-Bona22 | -4.791667e-01 | -2.66201504 | 1.703681704 | 1.0000000 |
| Degumille27-Bona22 | 1.437500e+00 | -0.86670890 | 3.741708902 | 0.7978949 |
| Degumille44-Bona22 | -1.062500e+00 | -3.15435600 | 1.029356003 | 0.9672260 |
| Degumille9-Bona22 | -1.705357e+00 | -3.79721315 | 0.386498860 | 0.2928212 |
| Bona7-Bona5 | 1.083333e+00 | -1.04690855 | 3.213575217 | 0.9667922 |
| Degumille27-Bona5 | 3.000000e+00 | 0.74556390 | 5.254436103 | 0.0005490*** |
| Degumille44-Bona5 | 5.000000e-01 | -1.53690084 | 2.536900836 | 0.9999996 |
| Degumille9-Bona5 | -1.428571e-01 | -2.17975798 | 1.894043693 | 1.0000000 |
| Degumille27-Bona7 | 1.916667e+00 | -0.53079492 | 4.364128258 | 0.3693556 |
| Degumille44-Bona7 | -5.833333e-01 | -2.83201097 | 1.665344303 | 0.9999989 |
| Degumille9-Bona7 | -1.226190e+00 | -3.47486811 | 1.022487160 | 0.9331120 |

Mean esterase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD3.

**Table C.28: Esterase activity of filaments compared between genotypes in stage FD1 (ES1)**

| Comparison FD1 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 2.228571e+00 | -1.604360970 | 6.061503827 | 0.8823773 |
| Bona22-Bona12 | 2.228571e+00 | -1.604360970 | 6.061503827 | 0.8823773 |
| Bona5-Bona12 | -2.857143e-01 | -3.784686846 | 3.213258274 | 1.0000000 |
| Bona7-Bona12 | 2.619048e-01 | -3.379941344 | 3.903750867 | 1.0000000 |
| Degumille27-Bona12 | -1.714286e-01 | -4.004360970 | 3.661503827 | 1.0000000 |
| Degumille44-Bona12 | 9.523810e-02 | -3.546608010 | 3.737084201 | 1.0000000 |
| Degumille9-Bona12 | 1.261905e+00 | -2.379941344 | 4.903750867 | 0.9998215 |
| Bona22-Bona19 | 6.661338e-15 | -4.140040165 | 4.140040165 | 1.0000000 |
| Bona5-Bona19 | -2.514286e+00 | -6.347218113 | 1.318646684 | 0.7199202 |
| Bona7-Bona19 | -1.966667e+00 | -5.930453348 | 1.997120014 | 0.9747335 |
| Degumille27-Bona19 | -2.400000e+00 | -6.540040165 | 1.740040165 | 0.8852436 |
| Degumille44-Bona19 | -2.133333e+00 | -6.097120014 | 1.830453348 | 0.9412266 |
| Degumille9-Bona19 | -9.666667e-01 | -4.930453348 | 2.997120014 | 0.9999997 |
| Bona5-Bona22 | -2.514286e+00 | -6.347218113 | 1.318646684 | 0.7199202 |
| Bona7-Bona22 | -1.966667e+00 | -5.930453348 | 1.997120014 | 0.9747335 |
| Degumille27-Bona22 | -2.400000e+00 | -6.540040165 | 1.740040165 | 0.8852436 |
| Degumille44-Bona22 | -2.133333e+00 | -6.097120014 | 1.830453348 | 0.9412266 |
| Degumille9-Bona22 | -9.666667e-01 | -4.930453348 | 2.997120014 | 0.9999997 |
| Bona7-Bona5 | 5.476190e-01 | -3.094227058 | 4.189465153 | 1.0000000 |
| Degumille27-Bona5 | 1.142857e-01 | -3.718646684 | 3.947218113 | 1.0000000 |
| Degumille44-Bona5 | 3.809524e-01 | -3.260893725 | 4.022798486 | 1.0000000 |
| Degumille9-Bona5 | 1.547619e+00 | -2.094227058 | 5.189465153 | 0.9962564 |
| Degumille27-Bona7 | -4.333333e-01 | -4.397120014 | 3.530453348 | 1.0000000 |
| Degumille44-Bona7 | -1.666667e-01 | -3.945988979 | 3.612655645 | 1.0000000 |
| Degumille9-Bona7 | 1.000000e+00 | -2.779322312 | 4.779322312 | 0.9999984 |
| Degumille44-Degumille27 | 2.666667e-01 | -3.697120014 | 4.230453348 | 1.0000000 |
| Degumille9-Degumille27 | 1.433333e+00 | -2.530453348 | 5.397120014 | 0.9996476 |

Mean esterase activity of filaments (fi) compared between the individual genotypes in stage FD1.

**Table C.29: Esterase activity of filaments compared between genotypes in stage FD2 (*ES1*)**

| Comparison FD2 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 4.285714e-01 | -3.070401131 | 3.927543988 | 1.0000000 |
| Bona22-Bona12 | 2.600000e+00 | -0.625893307 | 5.825893307 | 0.3142388 |
| Bona5-Bona12 | 5.000000e-01 | -2.887865613 | 3.887865613 | 1.0000000 |
| Bona7-Bona12 | 3.166667e+00 | -0.475179439 | 6.808512772 | 0.1875107 |
| Degumille27-Bona12 | 4.285714e-01 | -3.070401131 | 3.927543988 | 1.0000000 |
| Degumille44-Bona12 | 8.333333e-01 | -2.808512772 | 4.475179439 | 0.9999999 |
| Degumille9-Bona12 | 2.500000e+00 | -1.141846106 | 6.141846106 | 0.6371652 |
| Bona22-Bona19 | 2.171429e+00 | -1.054464736 | 5.397321879 | 0.6740851 |
| Bona5-Bona19 | 7.142857e-02 | -3.316437042 | 3.459294185 | 1.0000000 |
| Bona7-Bona19 | 2.738095e+00 | -0.903750867 | 6.379941344 | 0.4525572 |
| Degumille27-Bona19 | 2.220446e-15 | -3.498972560 | 3.498972560 | 1.0000000 |
| Degumille44-Bona19 | 4.047619e-01 | -3.237084201 | 4.046608010 | 1.0000000 |
| Degumille9-Bona19 | 2.071429e+00 | -1.570417534 | 5.713274677 | 0.9023939 |
| Bona5-Bona22 | -2.100000e+00 | -5.205030124 | 1.005030124 | 0.6653220 |
| Bona7-Bona22 | 5.666667e-01 | -2.813661973 | 3.946995306 | 1.0000000 |
| Degumille27-Bona22 | -2.171429e+00 | -5.397321879 | 1.054464736 | 0.6740851 |
| Degumille44-Bona22 | -1.766667e+00 | -5.146995306 | 1.613661973 | 0.9560896 |
| Degumille9-Bona22 | -1.000000e-01 | -3.480328639 | 3.280328639 | 1.0000000 |
| Bona7-Bona5 | 2.666667e+00 | -0.868565645 | 6.201898978 | 0.4457942 |
| Degumille27-Bona5 | -7.142857e-02 | -3.459294185 | 3.316437042 | 1.0000000 |
| Degumille44-Bona5 | 3.333333e-01 | -3.201898978 | 3.868565645 | 1.0000000 |
| Degumille9-Bona5 | 2.000000e+00 | -1.535232311 | 5.535232311 | 0.9068732 |
| Degumille27-Bona7 | -2.738095e+00 | -6.379941344 | 0.903750867 | 0.4525572 |
| Degumille44-Bona7 | -2.333333e+00 | -6.112655645 | 1.445988979 | 0.8127975 |
| Degumille9-Bona7 | -6.666667e-01 | -4.445988979 | 3.112655645 | 1.0000000 |

Mean esterase activity of filaments (fi) compared between the individual genotypes in stage FD2.

**Table C.30: Esterase activity of filaments compared between genotypes in stage FD3 (*ES1*)**

| Comparison FD3 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona19-Bona12 | 1.333333e+00 | -2.201898978 | 4.868565645 | 0.9993253 |
| Bona22-Bona12 | 2.375000e+00 | -0.897989131 | 5.647989131 | 0.5259499 |
| Bona5-Bona12 | -6.666667e-01 | -3.847440363 | 2.514107029 | 1.0000000 |
| Bona7-Bona12 | 1.500000e+00 | -2.035232311 | 5.035232311 | 0.9963336 |
| Degumille27-Bona12 | 2.800000e+00 | -0.931781774 | 6.531781774 | 0.4567668 |
| Degumille44-Bona12 | 1.428571e+00 | -1.959294185 | 4.816437042 | 0.9966304 |
| Degumille9-Bona12 | 1.285714e+00 | -2.102151328 | 4.673579899 | 0.9992588 |
| Bona22-Bona19 | 1.041667e+00 | -2.493565645 | 4.576898978 | 0.9999888 |
| Bona5-Bona19 | -2.000000e+00 | -5.450033471 | 1.450033471 | 0.8852436 |
| Bona7-Bona19 | 1.666667e-01 | -3.612655645 | 3.945988979 | 1.0000000 |
| Degumille27-Bona19 | 1.466667e+00 | -2.497120014 | 5.430453348 | 0.9994962 |
| Degumille44-Bona19 | 9.523810e-02 | -3.546608010 | 3.737084201 | 1.0000000 |
| Degumille9-Bona19 | -4.761905e-02 | -3.689465153 | 3.594227058 | 1.0000000 |
| Bona5-Bona22 | -3.041667e+00 | -6.222440363 | 0.139107029 | 0.0807122 |
| Bona7-Bona22 | -8.750000e-01 | -4.410232311 | 2.660232311 | 0.9999996 |
| Degumille27-Bona22 | 4.250000e-01 | -3.306781774 | 4.156781774 | 1.0000000 |
| Degumille44-Bona22 | -9.464286e-01 | -4.334294185 | 2.441437042 | 0.9999957 |
| Degumille9-Bona22 | -1.089286e+00 | -4.477151328 | 2.298579899 | 0.9999485 |
| Bona7-Bona5 | 2.166667e+00 | -1.283366804 | 5.616700137 | 0.7889122 |
| Degumille27-Bona5 | 3.466667e+00 | -0.184505565 | 7.117838898 | 0.0866828 |
| Degumille44-Bona5 | 2.095238e+00 | -1.203624871 | 5.394101061 | 0.7721177 |
| Degumille9-Bona5 | 1.952381e+00 | -1.346482013 | 5.251243918 | 0.8641363 |
| Degumille27-Bona7 | 1.300000e+00 | -2.663786681 | 5.263786681 | 0.9999279 |
| Degumille44-Bona7 | -7.142857e-02 | -3.713274677 | 3.570417534 | 1.0000000 |
| Degumille9-Bona7 | -2.142857e-01 | -3.856131820 | 3.427560391 | 1.0000000 |

Mean esterase activity of filaments (fi) compared between the individual genotypes in stage FD3.

# Appendix C.3: Post-hoc comparisons of test series *PE0*

**Table C.31: Comparison between the individual stages of floral development (*PE0*)**

| **Papillae** | | | | |
|---|---|---|---|---|
| Comparison | diff | Lwr | upr | p adj |
| FD2-FD1 | 0.7124304 | 0.1911094 | 1.233751 | 0.0042946** |
| FD3-FD1 | 2.2283298 | 1.6900759 | 2.766584 | 0.0000000*** |
| FD3-FD2 | 1.5158994 | 0.9913942 | 2.040405 | 0.0000000*** |
| **Pseudo-Papillae** | | | | |
| Comparison | diff | Lwr | upr | p adj |
| FD2-FD1 | 0.6467996 | 0.1060190 | 1.187580 | 0.0145666* |
| FD3-FD1 | 1.8549154 | 1.2965698 | 2.413261 | 0.0000000*** |
| FD3-FD2 | 1.2081158 | 0.6640321 | 1.752199 | 0.0000017*** |
| **Filament** | | | | |
| Comparison | diff | Lwr | upr | p adj |
| FD2-FD1 | 0.5714286 | - 0.1803651 | 1.323222 | 0.1730179 |
| FD3-FD1 | 1.6046512 | 0.8281540 | 2.381148 | 0.0000082*** |
| FD3-FD2 | 1.0332226 | 0.2723698 | 1.794075 | 0.0045790** |

Mean peroxidase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) compared between the individual stages of floral development (FD1, FD2, FD3).

**Table C.32: Comparison between self-compatible and self-incompatible genotypes (*PE0*)**

| SI-SC | Papillae | Pseudo-Papillae | Filament |
|---|---|---|---|
| diff | 0.3431694 | 0.04262295 | - 0.4967645 |
| lwr | - 0.1299303 | - 0.4114089 | - 1.056526 |
| upr | 0.8162691 | 0.4966548 | 0.06299755 |
| p adj | 0.1537185 | 0.8529831 | 0.0815079 |

Mean peroxidase activity of papillae, pseudo-papillae and filaments compared between self-compatible (SC) and (SI) genotypes.

**Table C.33: Comparison between SC and SI genotypes at different stages of floral development (*PE0*)**

| **Papillae** | | | | |
|---|---|---|---|---|
| SI-SC | diff | lwr | upr | p adj |
| FD1 | 1.15527950 | 0.2703575 | 2.0402015 | 0.0032310** |
| FD2 | - 0.27441077 | - 1.1164980 | 0.5676764 | 0.9345882 |
| FD3 | - 0.06333333 | - 0.9696505 | 0.8429838 | 0.9999525 |
| **Pseudo-Papillae** | | | | |
| SI-SC | diff | lwr | upr | p adj |
| FD1 | 0.7277433 | - 0.211965802 | 1.6674523 | 0.2268064 |
| FD2 | - 0.2491582 | - 1.143380485 | 0.6450640 | 0.9659777 |
| FD3 | - 0.5644444 | - 1.526873261 | 0.3979844 | 0.5365962 |
| **Filaments** | | | | |
| SI-SC | diff | lwr | upr | p adj |
| FD1 | 0.62500000 | - 0.62945095 | 1.8794510 | 0.7019392 |
| FD2 | - 0.61279461 | - 1.81857305 | 0.5929838 | 0.6839219 |
| FD3 | - 1.73111111 | - 3.02886001 | - 0.4333622 | 0.0024161** |

Mean peroxidase activity of papillae (top), pseudo-papillae (middle) and filaments (bottom) compared between self-compatible (SC) and (SI) genotypes at different floral development stages (FD1, FD2, FD3).

**Table C.34: Peroxidase activity of papillae compared between genotypes (*PE0*)**

| Comparison (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -0.90140500 | -1.6737588 | -0.12905120 | 0.0113876* |
| Bona5-Bona12 | -0.50557600 | -1.3197084 | 0.30855639 | 0.5084231 |
| Bona7-Bona12 | -0.84801788 | -1.7441326 | 0.04809688 | 0.0762530 |
| Degumille27-Bona12 | -0.24916467 | -1.0306591 | 0.53232972 | 0.9620150 |
| Degumille8-Bona12 | -0.36500920 | -1.1564363 | 0.42641793 | 0.8091772 |
| Degumille9-Bona12 | -0.21500920 | -1.0064363 | 0.57641793 | 0.9829588 |
| Bona5-Bona15 | 0.39582900 | -0.4183034 | 1.20996139 | 0.7680863 |
| Bona7-Bona15 | 0.05338711 | -0.8427277 | 0.94950188 | 0.9999971 |
| Degumille27-Bona15 | 0.65224033 | -0.1292541 | 1.43373472 | 0.1673113 |
| Degumille8-Bona15 | 0.53639580 | -0.2550313 | 1.32782293 | 0.3992453 |
| Degumille9-Bona15 | 0.68639580 | -0.1050313 | 1.47782293 | 0.1347613 |
| Bona7-Bona5 | -0.34244189 | -1.2748060 | 0.58992222 | 0.9262436 |
| Degumille27-Bona5 | 0.25641132 | -0.5663977 | 1.07922032 | 0.9660198 |
| Degumille8-Bona5 | 0.14056680 | -0.6916820 | 0.97281559 | 0.9987168 |
| Degumille9-Bona5 | 0.29056680 | -0.5416820 | 1.12281559 | 0.9415244 |
| Degumille27-Bona7 | 0.59885321 | -0.3051516 | 1.50285807 | 0.4278367 |
| Degumille8-Bona7 | 0.48300869 | -0.4295965 | 1.39561385 | 0.6899429 |
| Degumille9-Bona7 | 0.63300869 | -0.2795965 | 1.54561385 | 0.3704975 |
| Degumille8-Degumille27 | -0.11584453 | -0.9161945 | 0.68450540 | 0.9994682 |
| Degumille9-Degumille27 | 0.03415547 | -0.7661945 | 0.83450540 | 0.9999996 |
| Degumille9-Degumille8 | 0.15000000 | -0.6600515 | 0.96005150 | 0.9978467 |

Mean peroxidase activity of papillae (p) compared between the individual genotypes.

**Table C.35: Peroxidase activity of pseudo-papillae compared between genotypes (*PE0*)**

| Comparison (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -1.16560770 | -1.9144507 | -0.416764720 | 0.0001629*** |
| Bona5-Bona12 | -0.82033183 | -1.6096816 | -0.030982016 | 0.0361006* |
| Bona7-Bona12 | -1.09801398 | -1.9668506 | -0.229177368 | 0.0043641** |
| Degumille27-Bona12 | -0.92036759 | -1.6780729 | -0.162662248 | 0.0071651** |
| Degumille8-Bona12 | -1.13164932 | -1.8989850 | -0.364313604 | 0.0004301*** |
| Degumille9-Bona12 | -1.93164932 | -2.6989850 | -1.164313604 | 0.0000000*** |
| Bona5-Bona15 | 0.34527588 | -0.4440739 | 1.134625689 | 0.8447002 |
| Bona7-Bona15 | 0.06759372 | -0.8012429 | 0.936430337 | 0.9999860 |
| Degumille27-Bona15 | 0.24524012 | -0.5124652 | 1.002945457 | 0.9591494 |
| Degumille8-Bona15 | 0.03395838 | -0.7333773 | 0.801294101 | 0.9999995 |
| Degumille9-Bona15 | -0.76604162 | -1.5333773 | 0.001294101 | 0.0506886 |
| Bona7-Bona5 | -0.27768215 | -1.1816647 | 0.626300355 | 0.9683402 |
| Degumille27-Bona5 | -0.10003576 | -0.8977981 | 0.697726546 | 0.9997680 |
| Degumille8-Bona5 | -0.31131749 | -1.1182322 | 0.495597259 | 0.9081926 |
| Degumille9-Bona5 | -1.11131749 | -1.9182322 | -0.304402741 | 0.0013008** |
| Degumille27-Bona7 | 0.17764639 | -0.6988401 | 1.054132916 | 0.9964434 |
| Degumille8-Bona7 | -0.03363534 | -0.9184604 | 0.851189696 | 0.9999998 |
| Degumille9-Bona7 | -0.83363534 | -1.7184604 | 0.051189696 | 0.0787089 |
| Degumille8-Degumille27 | -0.21128174 | -0.9872686 | 0.564705167 | 0.9827635 |
| Degumille9-Degumille27 | -1.01128174 | -1.7872686 | -0.235294833 | 0.0028938** |
| Degumille9-Degumille8 | -0.80000000 | -1.5853931 | -0.014606852 | 0.0429245* |

Mean peroxidase activity of pseudo-papillae (fp) compared between the individual genotypes.

**Table C.36: Peroxidase activity of filaments compared between genotypes (*PE0*)**

| Comparison (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -0.05542132 | -1.15628024 | 1.045438e+00 | 0.9999989 |
| Bona5-Bona12 | 1.27084802 | 0.11044082 | 2.431255e+00 | 0.0221569* |
| Bona7-Bona12 | -0.47360779 | -1.75086691 | 8.036513e-01 | 0.9230752 |
| Degumille27-Bona12 | 0.09442551 | -1.01946178 | 1.208313e+00 | 0.9999767 |
| Degumille8-Bona12 | -1.12811839 | -2.25616311 | -7.367619e-05 | 0.0499735* |
| Degumille9-Bona12 | 0.78672103 | -0.32716626 | 1.900608e+00 | 0.3484195 |
| Bona5-Bona15 | 1.32626934 | 0.16586215 | 2.486677e+00 | 0.0143005* |
| Bona7-Bona15 | -0.41818646 | -1.69544559 | 8.590727e-01 | 0.9568653 |
| Degumille27-Bona15 | 0.14984683 | -0.96404046 | 1.263734e+00 | 0.9996517 |
| Degumille8-Bona15 | -1.07269707 | -2.20074179 | 5.534765e-02 | 0.0736300 |
| Degumille9-Bona15 | 0.84214235 | -0.27174494 | 1.956030e+00 | 0.2682218 |
| Bona7-Bona5 | -1.74445580 | -3.07338220 | -4.155294e-01 | 0.0026133** |
| Degumille27-Bona5 | -1.17642251 | -2.34919673 | -3.648282e-03 | 0.0487505* |
| Degumille8-Bona5 | -2.39896641 | -3.58519546 | -1.212737e+00 | 0.0000003*** |
| Degumille9-Bona5 | -0.48412698 | -1.65690121 | 6.886472e-01 | 0.8774780 |
| Degumille27-Bona7 | 0.56803330 | -0.72047181 | 1.856538e+00 | 0.8399212 |
| Degumille8-Bona7 | -0.65451061 | -1.95527399 | 6.462528e-01 | 0.7384007 |
| Degumille9-Bona7 | 1.26032882 | -0.02817629 | 2.548834e+00 | 0.0595823 |
| Degumille8-Degumille27 | -1.22254390 | -2.36330655 | -8.178125e-02 | 0.0272868* |
| Degumille9-Degumille27 | 0.69229552 | -0.43446951 | 1.819061e+00 | 0.5216911 |
| Degumille9-Degumille8 | 1.91483942 | 0.77407678 | 3.055602e+00 | 0.0000356*** |

Mean peroxidase activity of filaments (fi) compared between the individual genotypes.

**Table C.37: Peroxidase activity of papillae compared between genotypes in stage FD1 (*PE0*)**

| Comparison FD1 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -1.564286e+00 | -3.10163701 | -0.02693442 | 0.0412266* |
| Bona5-Bona12 | -1.814286e+00 | -3.64093124 | 0.01235981 | 0.0537999 |
| Bona7-Bona12 | -7.142857e-01 | -2.66959293 | 1.24102150 | 0.9989823 |
| Degumille27-Bona12 | -1.130952e+00 | -2.86653266 | 0.60462790 | 0.6962537 |
| Degumille8-Bona12 | -1.630952e+00 | -3.36653266 | 0.10462790 | 0.0935553 |
| Degumille9-Bona12 | 7.857143e-01 | -0.94986600 | 2.52129457 | 0.9855151 |
| Bona5-Bona15 | -2.500000e-01 | -1.95867043 | 1.45867043 | 1.0000000 |
| Bona7-Bona15 | 8.500000e-01 | -0.99557500 | 2.69557500 | 0.9825241 |
| Degumille27-Bona15 | 4.333333e-01 | -1.17761660 | 2.04428326 | 0.9999898 |
| Degumille8-Bona15 | -6.666667e-02 | -1.67761660 | 1.54428326 | 1.0000000 |
| Degumille9-Bona15 | 2.350000e+00 | 0.73905007 | 3.96094993 | 0.0000953*** |
| Bona7-Bona5 | 1.100000e+00 | -0.99268535 | 3.19268535 | 0.9356579 |
| Degumille27-Bona5 | 6.833333e-01 | -1.20567290 | 2.57233957 | 0.9991099 |
| Degumille8-Bona5 | 1.833333e-01 | -1.70567290 | 2.07233957 | 1.0000000 |
| Degumille9-Bona5 | 2.600000e+00 | 0.71099376 | 4.48900624 | 0.0003416*** |
| Degumille27-Bona7 | -4.166667e-01 | -2.43035408 | 1.59702075 | 0.9999999 |
| Degumille8-Bona7 | -9.166667e-01 | -2.93035408 | 1.09702075 | 0.9846077 |
| Degumille9-Bona7 | 1.500000e+00 | -0.51368741 | 3.51368741 | 0.4445372 |
| Degumille8-Degumille27 | -5.000000e-01 | -2.30109678 | 1.30109678 | 0.9999830 |
| Degumille9-Degumille27 | 1.916667e+00 | 0.11556989 | 3.71776344 | 0.0241261* |
| Degumille9-Degumille8 | 2.416667e+00 | 0.61556989 | 4.21776344 | 0.0005746*** |

Mean peroxidase activity of papillae (p) compared between the individual genotypes in stage FD1.

**Table C.38: Peroxidase activity of papillae compared between genotypes in stage FD2 (*PE0*)**

| Comparison FD2 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -1.488095e+00 | -3.22367552 | 0.24748504 | 0.1985397 |
| Bona5-Bona12 | 2.857143e-01 | -1.38177731 | 1.95320588 | 1.0000000 |
| Bona7-Bona12 | -1.154762e+00 | -2.89034219 | 0.58081838 | 0.6602845 |
| Degumille27-Bona12 | -1.825397e-01 | -1.75466584 | 1.38958647 | 1.0000000 |
| Degumille8-Bona12 | -3.996803e-15 | -1.66749160 | 1.66749160 | 1.0000000 |
| Degumille9-Bona12 | -1.571429e+00 | -3.23892017 | 0.09606303 | 0.0911073 |
| Bona5-Bona15 | 1.773810e+00 | 0.03822924 | 3.50938981 | 0.0392010* |
| Bona7-Bona15 | 3.333333e-01 | -1.46776344 | 2.13443011 | 1.0000000 |
| Degumille27-Bona15 | 1.305556e+00 | -0.33861333 | 2.94972444 | 0.3233951 |
| Degumille8-Bona15 | 1.488095e+00 | -0.24748504 | 3.22367552 | 0.1985397 |
| Degumille9-Bona15 | -8.333333e-02 | -1.81891362 | 1.65224695 | 1.0000000 |
| Bona7-Bona5 | -1.440476e+00 | -3.17605647 | 0.29510409 | 0.2477986 |
| Degumille27-Bona5 | -4.682540e-01 | -2.04038012 | 1.10387219 | 0.9999484 |
| Degumille8-Bona5 | -2.857143e-01 | -1.95320588 | 1.38177731 | 1.0000000 |
| Degumille9-Bona5 | -1.857143e+00 | -3.52463445 | -0.18965126 | 0.0132109* |
| Degumille27-Bona7 | 9.722222e-01 | -0.67194667 | 2.61639111 | 0.8352620 |
| Degumille8-Bona7 | 1.154762e+00 | -0.58081838 | 2.89034219 | 0.6602845 |
| Degumille9-Bona7 | -4.166667e-01 | -2.15224695 | 1.31891362 | 0.9999985 |
| Degumille8-Degumille27 | 1.825397e-01 | -1.38958647 | 1.75466584 | 1.0000000 |
| Degumille9-Degumille27 | -1.388889e+00 | -2.96101504 | 0.18323727 | 0.1586512 |
| Degumille9-Degumille8 | -1.571429e+00 | -3.23892017 | 0.09606303 | 0.0911073 |

Mean peroxidase activity of papillae (p) compared between the individual genotypes in stage FD2.

**Table C.39: Peroxidase activity of papillae compared between genotypes in stage FD3 (*PE0*)**

| Comparison FD3 (p) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 3.958333e-01 | -1.28893843 | 2.08060510 | 0.9999989 |
| Bona5-Bona12 | -3.541667e-01 | -2.03893843 | 1.33060510 | 0.9999998 |
| Bona7-Bona12 | -7.708333e-01 | -2.88280651 | 1.34113984 | 0.9989945 |
| Degumille27-Bona12 | 3.958333e-01 | -1.28893843 | 2.08060510 | 0.9999989 |
| Degumille8-Bona12 | 3.482143e-01 | -1.26632751 | 1.96275608 | 0.9999998 |
| Degumille9-Bona12 | 2.767857e-01 | -1.33775608 | 1.89132751 | 1.0000000 |
| Bona5-Bona15 | -7.500000e-01 | -2.55109678 | 1.05109678 | 0.9944980 |
| Bona7-Bona15 | -1.166667e+00 | -3.37255071 | 1.03921737 | 0.9320550 |
| Degumille27-Bona15 | 0.000000e+00 | -1.80109678 | 1.80109678 | 1.0000000 |
| Degumille8-Bona15 | -4.761905e-02 | -1.78319933 | 1.68796123 | 1.0000000 |
| Degumille9-Bona15 | -1.190476e-01 | -1.85462790 | 1.61653266 | 1.0000000 |
| Bona7-Bona5 | -4.166667e-01 | -2.62255071 | 1.78921737 | 1.0000000 |
| Degumille27-Bona5 | 7.500000e-01 | -1.05109678 | 2.55109678 | 0.9944980 |
| Degumille8-Bona5 | 7.023810e-01 | -1.03319933 | 2.43796123 | 0.9961260 |
| Degumille9-Bona5 | 6.309524e-01 | -1.10462790 | 2.36653266 | 0.9990474 |
| Degumille27-Bona7 | 1.166667e+00 | -1.03921737 | 3.37255071 | 0.9320550 |
| Degumille8-Bona7 | 1.119048e+00 | -1.03367478 | 3.27177002 | 0.9417680 |
| Degumille9-Bona7 | 1.047619e+00 | -1.10510335 | 3.20034144 | 0.9689761 |
| Degumille8-Degumille27 | -4.761905e-02 | -1.78319933 | 1.68796123 | 1.0000000 |
| Degumille9-Degumille27 | -1.190476e-01 | -1.85462790 | 1.61653266 | 1.0000000 |
| Degumille9-Degumille8 | -7.142857e-02 | -1.73892017 | 1.59606303 | 1.0000000 |

Mean peroxidase activity of papillae (p) compared between the individual genotypes in stage FD3.

**Table C.40: Peroxidase activity of pseudo-papillae compared between genotypes in stage FD1 (*PE0*)**

| Comparison FD1 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -1.471429e+00 | -2.96198217 | 0.019125026 | 0.0574152 |
| Bona5-Bona12 | -2.071429e+00 | -3.84247015 | -0.300386993 | 0.0064556** |
| Bona7-Bona12 | -4.464286e-01 | -2.34221532 | 1.449358177 | 0.9999989 |
| Degumille27-Bona12 | -1.738095e+00 | -3.42084364 | -0.055346834 | 0.0346748* |
| Degumille8-Bona12 | -1.988095e+00 | -3.67084364 | -0.305346834 | 0.0055201** |
| Degumille9-Bona12 | -1.404762e+00 | -3.08751031 | 0.277986499 | 0.2385766 |
| Bona5-Bona15 | -6.000000e-01 | -2.25665770 | 1.056657701 | 0.9990952 |
| Bona7-Bona15 | 1.025000e+00 | -0.76439483 | 2.814394831 | 0.8694388 |
| Degumille27-Bona15 | -2.666667e-01 | -1.82857853 | 1.295245193 | 1.0000000 |
| Degumille8-Bona15 | -5.166667e-01 | -2.07857853 | 1.045245193 | 0.9997495 |
| Degumille9-Bona15 | 6.666667e-02 | -1.49524519 | 1.628578526 | 1.0000000 |
| Bona7-Bona5 | 1.625000e+00 | -0.40398302 | 3.653983023 | 0.3080613 |
| Degumille27-Bona5 | 3.333333e-01 | -1.49817067 | 2.164837334 | 1.0000000 |
| Degumille8-Bona5 | 8.333333e-02 | -1.74817067 | 1.914837334 | 1.0000000 |
| Degumille9-Bona5 | 6.666667e-01 | -1.16483733 | 2.498170667 | 0.9990308 |
| Degumille27-Bona7 | -1.291667e+00 | -3.24405649 | 0.660723158 | 0.6702602 |
| Degumille8-Bona7 | -1.541667e+00 | -3.49405649 | 0.410723158 | 0.3335254 |
| Degumille9-Bona7 | -9.583333e-01 | -2.91072316 | 0.994056491 | 0.9662002 |
| Degumille8-Degumille27 | -2.500000e-01 | -1.99627055 | 1.496270546 | 1.0000000 |
| Degumille9-Degumille27 | 3.333333e-01 | -1.41293721 | 2.079603880 | 1.0000000 |
| Degumille9-Degumille8 | 5.833333e-01 | -1.16293721 | 2.329603880 | 0.9997111 |

Mean peroxidase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD1.

**Table C.41: Peroxidase activity of pseudo-papillae compared between genotypes in stage FD2 (*PE0*)**

| Comparison FD2 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -1.940476e+00 | -3.62322459 | -0.257727786 | 0.0080019** |
| Bona5-Bona12 | 1.428571e-01 | -1.47387523 | 1.759589514 | 1.0000000 |
| Bona7-Bona12 | -1.690476e+00 | -3.37322459 | -0.007727786 | 0.0475558* |
| Degumille27-Bona12 | -8.015873e-01 | -2.32585720 | 0.722682596 | 0.9353938 |
| Degumille8-Bona12 | -3.571429e-01 | -1.97387523 | 1.259589514 | 0.9999996 |
| Degumille9-Bona12 | -2.285714e+00 | -3.90244666 | -0.668981914 | 0.0001926*** |
| Bona5-Bona15 | 2.083333e+00 | 0.40058493 | 3.766081737 | 0.0025577** |
| Bona7-Bona15 | 2.500000e-01 | -1.49627055 | 1.996270546 | 1.0000000 |
| Degumille27-Bona15 | 1.138889e+00 | -0.45523073 | 2.733008505 | 0.5265219 |
| Degumille8-Bona15 | 1.583333e+00 | -0.09941507 | 3.266081737 | 0.0924458 |
| Degumille9-Bona15 | -3.452381e-01 | -2.02798650 | 1.337510309 | 0.9999999 |
| Bona7-Bona5 | -1.833333e+00 | -3.51608174 | -0.150584929 | 0.0178089* |
| Degumille27-Bona5 | -9.444444e-01 | -2.46871434 | 0.579825453 | 0.7747215 |
| Degumille8-Bona5 | -5.000000e-01 | -2.11673237 | 1.116732372 | 0.9999081 |
| Degumille9-Bona5 | -2.428571e+00 | -4.04530380 | -0.811839057 | 0.0000477*** |
| Degumille27-Bona7 | 8.888889e-01 | -0.70523073 | 2.483008505 | 0.8940994 |
| Degumille8-Bona7 | 1.333333e+00 | -0.34941507 | 3.016081737 | 0.3272641 |
| Degumille9-Bona7 | -5.952381e-01 | -2.27798650 | 1.087510309 | 0.9993472 |
| Degumille8-Degumille27 | 4.444444e-01 | -1.07982545 | 1.968714342 | 0.9999630 |
| Degumille9-Degumille27 | -1.484127e+00 | -3.00839688 | 0.040142914 | 0.0662237 |
| Degumille9-Degumille8 | -1.928571e+00 | -3.54530380 | -0.311839057 | 0.0047394** |

Mean peroxidase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD2.

**Table C.42: Peroxidase activity of pseudo-papillae compared between genotypes in stage FD3 (*PE0*)**

| Comparison FD3 (fp) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -1.250000e-01 | -1.75848652 | 1.508486522 | 1.0000000 |
| Bona5-Bona12 | -8.750000e-01 | -2.50848652 | 0.758486522 | 0.9240965 |
| Bona7-Bona12 | -8.750000e-01 | -2.92268372 | 1.172683723 | 0.9925070 |
| Degumille27-Bona12 | -2.916667e-01 | -1.92515319 | 1.341819856 | 1.0000000 |
| Degumille8-Bona12 | -1.160714e+00 | -2.72610867 | 0.404680102 | 0.4535504 |
| Degumille9-Bona12 | -2.017857e+00 | -3.58325153 | -0.452462755 | 0.0012434** |
| Bona5-Bona15 | -7.500000e-01 | -2.49627055 | 0.996270546 | 0.9920461 |
| Bona7-Bona15 | -7.500000e-01 | -2.88873590 | 1.388735896 | 0.9994218 |
| Degumille27-Bona15 | -1.666667e-01 | -1.91293721 | 1.579603880 | 1.0000000 |
| Degumille8-Bona15 | -1.035714e+00 | -2.71846269 | 0.647034118 | 0.7840986 |
| Degumille9-Bona15 | -1.892857e+00 | -3.57560555 | -0.210108739 | 0.0114901* |
| Bona7-Bona5 | 2.220446e-15 | -2.13873590 | 2.138735896 | 1.0000000 |
| Degumille27-Bona5 | 5.833333e-01 | -1.16293721 | 2.329603880 | 0.9997111 |
| Degumille8-Bona5 | -2.857143e-01 | -1.96846269 | 1.397034118 | 1.0000000 |
| Degumille9-Bona5 | -1.142857e+00 | -2.82560555 | 0.539891261 | 0.6231304 |
| Degumille27-Bona7 | 5.833333e-01 | -1.55540256 | 2.722069229 | 0.9999872 |
| Degumille8-Bona7 | -2.857143e-01 | -2.37290680 | 1.801478231 | 1.0000000 |
| Degumille9-Bona7 | -1.142857e+00 | -3.23004966 | 0.944335374 | 0.9086480 |
| Degumille8-Degumille27 | -8.690476e-01 | -2.55179602 | 0.813700785 | 0.9451304 |
| Degumille9-Degumille27 | -1.726190e+00 | -3.40893888 | -0.043442072 | 0.0375663* |
| Degumille9-Degumille8 | -8.571429e-01 | -2.47387523 | 0.759589514 | 0.9305960 |

Mean peroxidase activity of pseudo-papillae (fp) compared between the individual genotypes in stage FD3.

**Table C.43: Peroxidase activity of filaments compared between genotypes in stage FD1 (*PE0*)**

| Comparison FD1 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -1.171429e+00 | -3.36255146 | 1.01969432 | 0.9255455 |
| Bona5-Bona12 | -5.714286e-01 | -3.17487051 | 2.03201337 | 0.9999997 |
| Bona7-Bona12 | -1.071429e+00 | -3.85824667 | 1.71538953 | 0.9979971 |
| Degumille27-Bona12 | -7.380952e-01 | -3.21174567 | 1.73555519 | 0.9999473 |
| Degumille8-Bona12 | -1.238095e+00 | -3.71174567 | 1.23555519 | 0.9592393 |
| Degumille9-Bona12 | 7.142857e-01 | -1.66232075 | 3.09089217 | 0.9999411 |
| Bona5-Bona15 | 6.000000e-01 | -1.83529694 | 3.03529694 | 0.9999976 |
| Bona7-Bona15 | 1.000000e-01 | -2.53042133 | 2.73042133 | 1.0000000 |
| Degumille27-Bona15 | 4.333333e-01 | -1.86268664 | 2.72935331 | 1.0000000 |
| Degumille8-Bona15 | -6.666667e-02 | -2.36268664 | 2.22935331 | 1.0000000 |
| Degumille9-Bona15 | 1.885714e+00 | -0.30540861 | 4.07683718 | 0.1933858 |
| Bona7-Bona5 | -5.000000e-01 | -3.48261744 | 2.48261744 | 1.0000000 |
| Degumille27-Bona5 | -1.666667e-01 | -2.85898873 | 2.52565540 | 1.0000000 |
| Degumille8-Bona5 | -6.666667e-01 | -3.35898873 | 2.02565540 | 0.9999974 |
| Degumille9-Bona5 | 1.285714e+00 | -1.31772765 | 3.88915622 | 0.9642195 |
| Degumille27-Bona7 | 3.333333e-01 | -2.53669163 | 3.20335830 | 1.0000000 |
| Degumille8-Bona7 | -1.666667e-01 | -3.03669163 | 2.70335830 | 1.0000000 |
| Degumille9-Bona7 | 1.785714e+00 | -1.00110381 | 4.57253239 | 0.7241964 |
| Degumille8-Degumille27 | -5.000000e-01 | -3.06702837 | 2.06702837 | 1.0000000 |
| Degumille9-Degumille27 | 1.452381e+00 | -1.02126948 | 3.92603138 | 0.8436610 |
| Degumille9-Degumille8 | 1.952381e+00 | -0.52126948 | 4.42603138 | 0.3345963 |

Mean peroxidase activity of filaments (fi) compared between the individual genotypes in stage FD1.

**Table C.44: Peroxidase activity of filaments compared between genotypes in stage FD2 (*PE0*)**

| Comparison FD2 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | -3.809524e-01 | -2.85460281 | 2.09269805 | 1.0000000 |
| Bona5-Bona12 | 2.714286e+00 | 0.33767925 | 5.09089217 | 0.0092176** |
| Bona7-Bona12 | 1.285714e+00 | -1.18793615 | 3.75936472 | 0.9419936 |
| Degumille27-Bona12 | 1.063492e+00 | -1.17719400 | 3.30417812 | 0.9760477 |
| Degumille8-Bona12 | -4.285714e-01 | -2.80517789 | 1.94803503 | 1.0000000 |
| Degumille9-Bona12 | 1.571429e+00 | -0.80517789 | 3.94803503 | 0.6716225 |
| Bona5-Bona15 | 3.095238e+00 | 0.62158766 | 5.56888853 | 0.0021205** |
| Bona7-Bona15 | 1.666667e+00 | -0.90036170 | 4.23369504 | 0.7027121 |
| Degumille27-Bona15 | 1.444444e+00 | -0.89892113 | 3.78781002 | 0.7823830 |
| Degumille8-Bona15 | -4.761905e-02 | -2.52126948 | 2.42603138 | 1.0000000 |
| Degumille9-Bona15 | 1.952381e+00 | -0.52126948 | 4.42603138 | 0.3345963 |
| Bona7-Bona5 | -1.428571e+00 | -3.90222186 | 1.04507900 | 0.8614663 |
| Degumille27-Bona5 | -1.650794e+00 | -3.89147971 | 0.58989241 | 0.4664852 |
| Degumille8-Bona5 | -3.142857e+00 | -5.51946360 | -0.76625068 | 0.0007621*** |
| Degumille9-Bona5 | -1.142857e+00 | -3.51946360 | 1.23374932 | 0.9725934 |
| Degumille27-Bona7 | -2.222222e-01 | -2.56558779 | 2.12114335 | 1.0000000 |
| Degumille8-Bona7 | -1.714286e+00 | -4.18793615 | 0.75936472 | 0.5856516 |
| Degumille9-Bona7 | 2.857143e-01 | -2.18793615 | 2.75936472 | 1.0000000 |
| Degumille8-Degumille27 | -1.492063e+00 | -3.73274955 | 0.74862257 | 0.6591941 |
| Degumille9-Degumille27 | 5.079365e-01 | -1.73274955 | 2.74862257 | 0.9999994 |
| Degumille9-Degumille8 | 2.000000e+00 | -0.37660646 | 4.37660646 | 0.2262675 |

Mean peroxidase activity of filaments (fi) compared between the individual genotypes in stage FD2.

**Table C.45: Peroxidase activity of filaments compared between genotypes in stage FD3 (*PE0*)**

| Comparison FD3 (fi) | diff | upr | lwr | p adj |
|---|---|---|---|---|
| Bona15-Bona12 | 1.708333e+00 | -0.69290183 | 4.10956850 | 0.5350662 |
| Bona5-Bona12 | 1.375000e+00 | -1.02623516 | 3.77623516 | 0.8700446 |
| Bona7-Bona12 | -2.625000e+00 | -5.63510758 | 0.38510758 | 0.1753853 |
| Degumille27-Bona12 | -1.250000e-01 | -2.52623516 | 2.27623516 | 1.0000000 |
| Degumille8-Bona12 | -1.625000e+00 | -3.92613931 | 0.67613931 | 0.5495207 |
| Degumille9-Bona12 | 8.928571e-02 | -2.21185360 | 2.39042502 | 1.0000000 |
| Bona5-Bona15 | -3.333333e-01 | -2.90036170 | 2.23369504 | 1.0000000 |
| Bona7-Bona15 | -4.333333e+00 | -7.47728816 | -1.18937850 | 0.0003301*** |
| Degumille27-Bona15 | -1.833333e+00 | -4.40036170 | 0.73369504 | 0.5275453 |
| Degumille8-Bona15 | -3.333333e+00 | -5.80698376 | -0.85968290 | 0.0005250*** |
| Degumille9-Bona15 | -1.619048e+00 | -4.09269805 | 0.85460281 | 0.6891404 |
| Bona7-Bona5 | -4.000000e+00 | -7.14395483 | -0.85604517 | 0.0015755** |
| Degumille27-Bona5 | -1.500000e+00 | -4.06702837 | 1.06702837 | 0.8489808 |
| Degumille8-Bona5 | -3.000000e+00 | -5.47365043 | -0.52634957 | 0.0036135** |
| Degumille9-Bona5 | -1.285714e+00 | -3.75936472 | 1.18793615 | 0.9419936 |
| Degumille27-Bona7 | 2.500000e+00 | -0.64395483 | 5.64395483 | 0.3210750 |
| Degumille8-Bona7 | 1.000000e+00 | -2.06818575 | 4.06818575 | 0.9997997 |
| Degumille9-Bona7 | 2.714286e+00 | -0.35390003 | 5.78247146 | 0.1570464 |
| Degumille8-Degumille27 | -1.500000e+00 | -3.97365043 | 0.97365043 | 0.8044915 |
| Degumille9-Degumille27 | 2.142857e-01 | -2.25936472 | 2.68793615 | 1.0000000 |
| Degumille9-Degumille8 | 1.714286e+00 | -0.66232075 | 4.09089217 | 0.5080763 |

Mean peroxidase activity of filaments (fi) compared between the individual genotypes in stage FD3.

Submitted by:

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