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Effects of Seed Injury and Injection with Gibberellic Acid and Paclobutrazol on Fruit Drop and Seed Development in 'Beni Shimizu' Peach

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The relationships between fruit drop induced by seed injury and seed development were investigated in 'Beni Shimizu' peach. Seed injury (Injury) was done by drilling from opposite side of suture line upto seed coat using an electric drill (outer diameter of 2.5 mm) at 40, 50, 60, 72 and 80 days after full bloom (DAFB). Injections of gibberellic acid (GA) and paclobutrazol (PBZ) (Injection), which relate to fruit drop and seed development were carried out just after the injury for seed. In all of the Injury and Injection treatments, treated fruit dropped in 40, 50 and 60 DAFB treatments but not in 72 and 80 DAFB treatments. Weight of fruit and seed decreased rapidly at 3 days after treatment (DAT) in 60 DAFB treatment. Morphology of endosperm and embryo was compared among 60, 72 and 80 DAFB treatments. Growth of endosperm and embryo stopped from 3 DAT in 60 DAFB treatment but embryo continued to grow until 5 DAT in 72 DAFB treatment. Collapsed cell nucleus in endosperm and embryo occurred at 3 DAT in 60 DAFB treatment. On the other hand, in 72 DAFB treatment cell nuclei in endosperm collapsed in a similar manner as in 60 DAFB treatment although the cell nuclei in embryo did not collapse. When morphology of chalazal haustorium in 60 DAFB treatment was examined, chalazal haustorium began to shrink just after the treatment and percentage of seed with normal chalazal haustorium decreased rapidly in all treatments. Based on these results, the process of seed abortion as a result of seed injury and its relationship between embryo growth and fruit drop is discussed.

Key words : fruit drop, injection of gibberellic acid, peach, seed development, seed injury

Introduction

In peach (*Prunus persica* Batsch) growing, physiological fruit drop which occurs from mid Growth Stage 2 to beginning of Growth Stage 3 is one of most important physiological disorders. Among the factors that increase physiological fruit drop in peach, seed abortion has been suggested as the cause of individual fruit drop[13]. The endosperm and embryo are important for fruit development as they are suppliers of such plant growth regulator as auxin and gibberellic acid[4,9]. It is well-known that in various fruit species gibberellic acid suppresses fruit drop and promotes fruit development[8]. Relationships between embryo growth and physiological fruit drop in peach have been suggested[2,14]. On the other hand, in order to clarify the process by which physiological fruit drop occurs, effect of artificial destruction of seed on fruit drop has been investigated[4,10,13,14]. These reports indicated that the normal and functional seed is needed to keep fruit development during Growth Stage 1 and 2. However the process with respect to seed abortion has almost never been described. This process needs to be understood in order to relate the process of seed abortion to physiological fruit drop.

The objective of this study was to examine the morphological change in seed abortion using injury and injection of gibberellic acid and paclobutrazol in 'Beni Shimizu' peach.

Materials and Methods

Plant materials

Three seven-year-old 'Beni Shimizu' peach trees grown in Field Science Center of the Faculty of Agriculture in Okayama University were used in 2004. Fruit were thinned at about 1.5-fold of standard fruit

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thinning level in early May.

Injury and Injection of gibberellic acid and paclobutrazol for peach seed

Using 110 fruit, drill (outer diameter: 2.5 mm) was inserted from the opposite side of the suture line of fruit so that drill injured the seed coat and nucellus only slightly in seed (Fig. 1). After drilling, the hole was immediately covered with Vaseline® (Injury). Using another 110 fruit, injection of gibberellic acid (GA) and paclobutrazol (PBZ) for the seed (GA or PBZ Injection) took place as followed. 0.1 ml aliquot of 500 ppm GA and PBZ was injected into the seed using a syringe just after drilling. After injection of GA or PBZ, the hole was covered with Vaseline®. Non-treated fruit were used as a control. All the treated fruit were covered with white paper bags.

Effect of Injury or GA and PBZ Injection on fruit drop

After the treatment, we confirmed whether treated fruit remained on the tree and date of beginning and end of fruit drop were recorded in each treatment. This investigation was done for 15 days after treatment and final percentage of drop fruit in each treatment was calculated.

Development of fruit and seed in Injury or GA and PBZ Injection fruit

In 60, 72 and 80 DAFB treatments, 12 non-treated fruit were collected just after treatment and 12 non-treated and treated fruit each were collected at 1, 3, 5 and 7 days after treatment (DAT). Seeds were taken out after measuring size and weight of fruit. The size and weight of seeds and degree of browning of seed coat were recorded. The degree of seed coat browning was assessed on a 4-point scale; 0: No browning of seed coat, 1: less than 50 % of seed coat is brown, 2: 50-100 % of seed coat is brown, and 3: 100 % of the area in seed coat is dark browned. After seeds were fixed with formalin acetic acid alcohol (FAA) fixative, length and morphology of endosperm and embryo were examined. Lengths of endosperm and embryo were measured with digital calipers after removing seed coat from seed cheek. Sections at apex of endosperm and embryo were prepared with microslicer at the thickness of 200-300 μm including one unbroken cell layer and stained in Giemsa stain solution for 5 minutes. Thereafter, sections were observed using light microscope. Existence of nucleus in 40 cells of endosperm and embryo was confirmed and the percentage of cell with collapsed cell nucleus in endosperm and embryo was calculated. The appearance of chalazal haustorium was confirmed as follows. Seed coat and nucellus near chalaza was removed so as not to break embryo sac and chalazal haustorium of endosperm was exposed. Chalazal hautorium was examined under a microscope and the percentage of seed with shrunken chalazal haustorium was calculated.

Results

The percentage of fruit drop and dates of beginning and end of fruit drop period in Injury and Injection treatments are shown in Table 1. In 40, 50 and 60 DAFB treatments, all treated fruit dropped but in 72 and 80 DAFB treatments, treated fruit did not drop even 18 days after treatment (DAT). In all Injury and Injection treatments, fruit drop began at 12 or 6 DAT and ended at 18 or 12 DAT in 40 or 50 and 60 DAFB treatments, respectively.

Changes in weight of fruit and seed in 60, 72 and 80 DAFB treatment are shown in Figure 2. In 60 DAFB

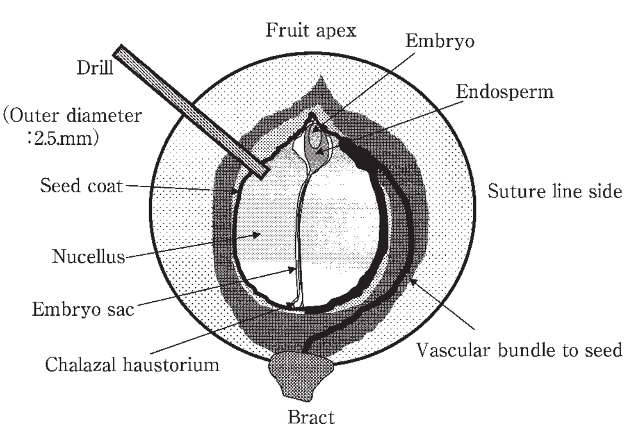

Fig. 1 Diagram of longitudinal cross section in peach fruit. Insertion point of drill in this experiment is shown.

Table 1 Effects of seed injury and injection with gibberellic acid (GA) and paclobutrazol on the percentage of dropped fruit and time of fruit drop in 'Beni Shimizu' peach

Treated date (DAFB[a])	Percentage of dropped fruit (%)			Time of Fruit drop (DAT[b])	
	Injury	GA	PBZ	Beginning	End
40	100	NT[c]	100	12	18
50	100	100	100	6	12
60	100	100	100	6	12
72	0	0	0	—[d]	—[d]
80	0	0	0	—[d]	—[d]

[a]Date of full bloom: April 6, 2004, DAFB; days after full bloom
[b]DAT; days after treatment
[c]NT; Not treated
[d]—: Fruit which were treated at 72 and 80 DAFB did not drop.

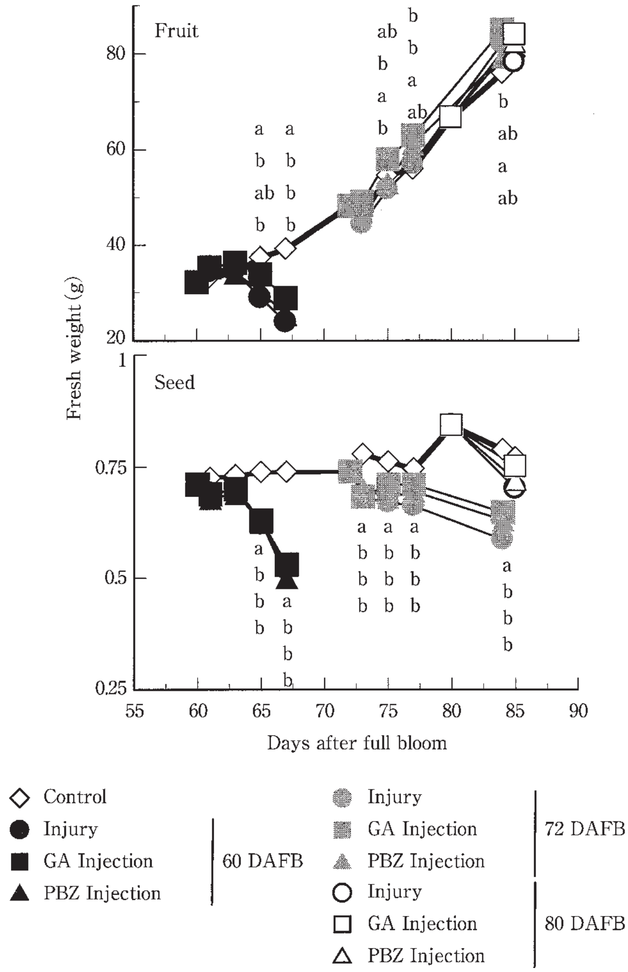

Fig. 2 Effects of seed injury and injections with gibberellic acid (GA) and paclobutrazol (PBZ) at 60, 72 and 80 days after full bloom (DAFB) on fruit (upper) and seed (lower) weights in 'Beni Shimizu' peach.

Letters of the alphabet indicate mean separation among treatments by Duncan's multiple range test at the 5% level (top: control, upper: seed injury, middle: GA Injection, lower: PBZ Injection).

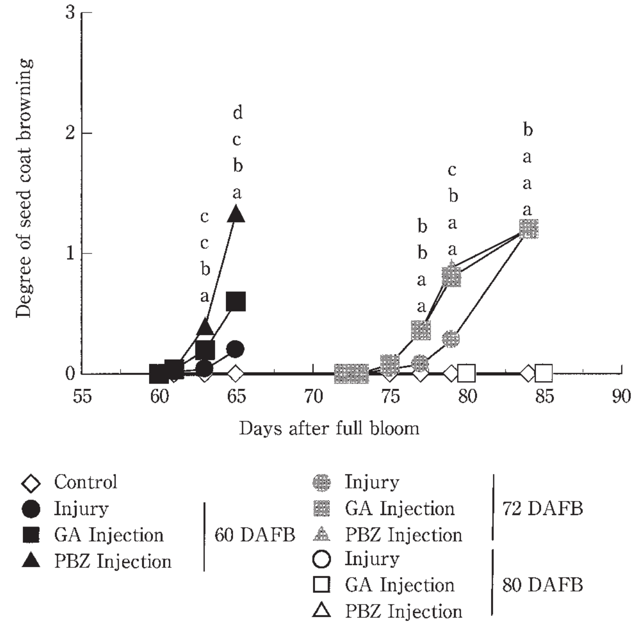

Fig. 3 Effects of seed injury and injections with gibberellic acid (GA) and paclobutrazol (PBZ) at 60, 72 and 80 days after full bloom (DAFB) on browning of seed coat in 'Beni Shimizu' peach.

Degree of seed coat browning was assessed using a scale of 4; 0: no browning of seed coat, 1: browning of less than 50% of the seed coat area, 2: 50–100% browning of the seed coat area, and 3: 100% of the seed coat area was dark brown. Letters of the alphabet indicate mean separation among treatments by Duncan's multiple range test at the 5% level (top: control, upper: seed injury, middle: GA Injection, lower: PBZ Injection).

treatment, weight of both fruit and seed decreased rapidly after 3 DAT. In 72 and 80 DAFB treatment, fruit weight was same as or higher than in control although seed weight decreased lightly at 3 DAT. In GA Injection, fruit weight was higher than in Injury and PBZ injection in 72 DAFB treatment. A similar pattern was observed in 60 DAFB treatment.

There was no difference in occurrence of browning of seed coat between 60 and 72 DAFB treatments (Fig. 3). In both treatments, browning of seed coat began to occur at 3 DAT and increased rapidly. In 60 DAFB treatment, browning of seed coat in PBZ injection was most severe. On the other hand, in 80 DAFB treatment no browning of seed coat occurred irrespective of the treatments.

Change in length of endosperm and embryo is shown in Fig. 4. In the control, mean endosperm length was about 10, 17 and 19 mm and embryo length was about 2.5, 8 and 16 mm at 60, 72 and 80 DAFB, respectively. The seed was filled with endosperm at 72 DAFB. In 60 DAFB treatment, endosperm stopped growing at 3 or 5 DAT in Injury and PBZ Injection or GA Injection, but in the 72 and 80 DAFB treatments there was no difference in endosperm length between treated and control fruit. On the other hand, embryo also stopped growing at 3 DAT at a length of less than 4 mm in 60 DAFB treatment. The embryo in GA Injection was a little larger than in Injury and PBZ Injection. In 72 DAFB treatment, there was almost no difference in embryo growth between treated and control fruit until 5 DAT but embryo in treated fruit almost stopped growing at 5 DAT. However, final embryo length reached about 12 mm in all Injury and Injection treatments. In 80 DAFB treatment, embryo growth was similar to that of the control.

In order to investigate morphological changes in cells of endosperm and embryo, occurrence of collap-

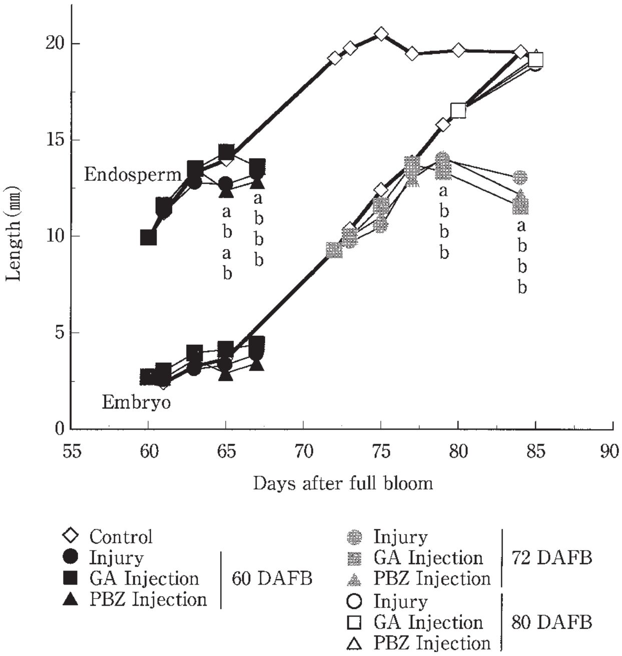

Fig. 4 Effects of seed injury and injection with gibberellic acid (GA) and paclobutrazol (PBZ) at 60, 72 and 80 days after full bloom (DAFB) on endosperm and embryo lengths in 'Beni Shimizu' peach.
Endosperms had attained full size at 72 days after full bloom. Letters of the alphabet indicate mean separation among treatments by Duncan's multiple range test at the 5% level (top: control, upper: seed injury, middle: GA Injection, lower: PBZ Injection).

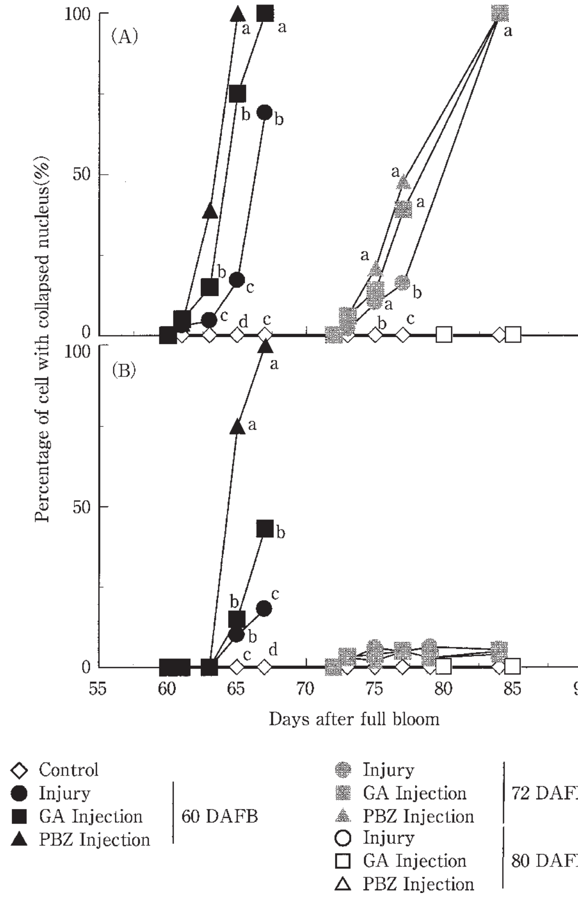

Fig. 5 Effects of seed injury and injection with gibberellic acid (GA) and paclobutrazol (PBZ) at 60, 72 and 80 days after full bloom (DAFB) on the percentage of cells with collapsed nucleus in endosperm (A) and embryo (B) in 'Beni Shimizu' peach.
Letters of the alphabet indicate mean separation among treatments by Duncan's multiple range test at the 5% level.

sed cell nucleus in endosperm and embryo was examined (Fig. 5). In the control, collapsing of cell nucleus did not occur in either endosperm or embryo during this investigation. In 60 DAFB treatment, the percentage of collapsed cell nucleus in endosperm increased rapidly from 3 DAT. In 72 DAFB treatment, although increase in collapsed cell nucleus was lower than in 60 DAFB treatment, percentage of collapsed cell nucleus became about 50% at 5 DAT in GA and PBZ Injection. There was a large difference in final percentage of collapsed cell nucleus in embryo between 60 and, 72 and 80 DAFB treatments. In 60 DAFB treatments collapsed cell nucleus increased more rapidly at 5 DAT in PBZ Injection than in other treatments and this was not observed in 72 and 80 DAFB.

When morphology of chalazal haustorium was investigated in 60 DAFB treatment, percentage of endosperm with normal chalazal haustorium in control fruit was constantly about 60% but decreased very rapidly after 1 DAT in both Injury and Injection fruit (Fig. 6). In almost all treated fruit chalazal haustorium did not function at 3 DAT.

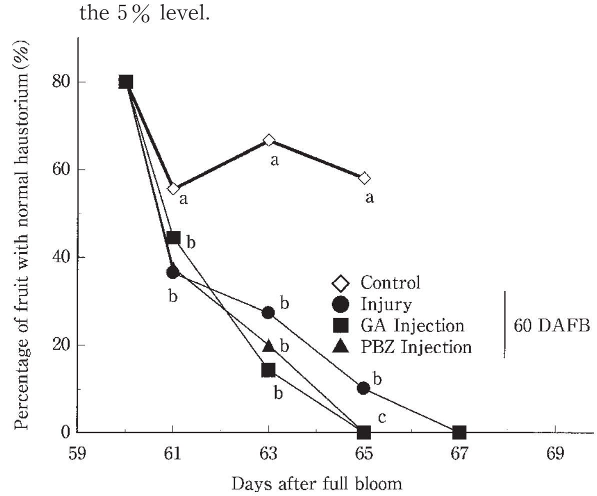

Fig. 6 Effects of seed injury and injection with gibberellic acid (GA) and paclobutrazol (PBZ) at 60 days after full bloom (DAFB) on the percentage of seed with normal chalazal haustorium in 'Beni Shimizu' peach.
Letters of the alphabet indicate mean separation among treatments by Duncan's multiple range test at the 5% level.

Discussion

In previous reports, the relationships between seed abortion and fruit development have been investigated using the destruction and removal of seed[6,10,13,14]. Loss of seed function induced fruit drop until pit-hardening stage and the seed was not needed in fruit development when fruit restarted their enlargement[6,14]. In this study, occurrence of fruit drop was entirely dependent on the time of injury to the seed; when the seed was injured before 60 DAFB, the fruit dropped from 6 to 15 DAT but fruit drilled after 72 DAFB did not drop until mature stage. Nakagawa et al.[6] reported that the fruit with the seed destroyed completely at 93 DAFB did not drop. However, in this study the treated fruit did not drop in 72 DAFB treatment. This indicates that injured seed may keep fruit development until seed is not needed for normal fruit enlargement in 72 DAFB treatment.

Morphological changes of endosperm and embryo in 60 DAFB treatment indicated a sequence of process in seed abortion under such stress conditions as injury to the seed. Peach has a non-albuminous seed[12]. Firstly, endosperm grows rapidly from beginning to mid Growth Stage 2. Secondly, substances reserved in endosperm are translocated to embryo and rapid embryo growth begins after about 1 week when rapid growth of endosperm occurs. Chalazal haustorium formed at apex of endosperm takes assimilates and mineral nutrients into the endosperm[11]. A relationship between abnormal symptom, not-reaching to chalaza, in chalazal haustorium and fruit drop in end of Growth Stage 1 was suggested[7]. Chalazal haustorium was easily affected by such stresses as injury because it shrunk at 1 DAT. Dittmann and Stösser[1] suggested that sink activity was inferior in the fruit with abnormal chalazal haustorium in Growth Stage 1. In 60 DAFB treatment the endosperm was immature and continued to grow until 3 DAT. It is considered that endosperm used up all the reserve in endosperm at 3 DAT because of broken chalazal haustorium, and translocation from endosperm and embryo also stopped. Therefore, embryo could not grow at 3 DAT. In both endosperm and embryo, cell nuclei collapsed rapidly. Collapse of cell nucleus in this immature tissue is one of the typical symptoms in necrosis[3]. Starvation, which occurs due to lack of translocation of assimilate to endosperm and embryo, may cause necrosis in endosperm or embryo. On the other hand, in 72 DAFB treatment, endosperm had already attained the full size just at treatment and reservation in endosperm might be supplied to embryo in longer periods than in 60 DAFB treatment even if translocation to endosperm through chalaza had stopped. Because the length when embryo stopped growing in 60 DAFB treatment almost coincided with embryo length in the fruit dropping owe to physiological fruit drop in 'Shimizu Hakuto', process of seed abortion may be supported by this hypothesis.

The differences in the seed components between 60 and 72 DAFB treatments were embryo length and percentage of collapsed cell nucleus in embryo. Fukuda et al.[2] suggested the size of embryo, when some factors which increase physiological fruit drop have occurred, affects the sustainability of fruit development in 'Shimizu Hakuto' peach. Wanaka[14] also suggested a close relationship between embryo size and fruit drop in an experiment using seed destruction, and treated fruit hardly dropped when embryo lengths were more than 5 mm. Embryos produce abundant GA during Growth Stage 2[4]. It is well known that GA is transported from seed to fruit and increases sink activity in the fruit and suppresses occurrence of physiological fruit drop in various fruit species[8]. It is plausible that there is a positive correlation between embryo length and GA content in the seed. Further, because necrosis hardly occurred in embryo cell in 72 DAFB treatment, embryo cells continued to produce plant growth regulator and embryos reached the size that embryo can keep normal fruit growth until 5 DAT when embryo growth stopped.

When seed was exposed to air owing to drilling, seed coat turned brown. Ethylene, which induces formation of abscission zone[10], emanates from the seed coat until Growth Stage 2 in air[5]. But the level of ethylene involved in seed coat rapidly decreased from end of Growth Stage 2 to Growth Stage 3. This tendency in ethylene evolution may induce the shrinking of chalazal haustorium in 60 DAFB treatment or decrease in collapsing of cell nucleus in embryo in 72 and 80 DAFB treatments. The causes inducing change in ethylene evolution in seed coat are not understood. Alternatively, ethylene evolution from seed coat may be suppressed by some substances produced in embryo after it reaches a certain size.

In order to clarify relationships between fluctuation of GA content in injured seed and fruit drop, injection of GA and PBZ into the seed was investigated. In this study, neither GA nor PBZ Injection affected the percentage of dropped fruit and duration of fruit drop period. It is plausible that embryos which stopped growing at 5 DAT in 72 DAFB treatment may be enough to maintain normal fruit growth until seed is not needed for fruit development. There is need to investigate GA and PBZ Injection during 60–72 DAFB in detail. Two issues regarding the relationship between injected GA and the development of fruit and seed were confirmed. In GA injection, fruit weight was higher at 60, 72, 80 DAFB treatments and lengths of endosperm and embryo were higher than Injury and PBZ Injection. These results may indicate that GA improves development of fruit and seed and GA injected to seed translocates to the flesh in GA Injection fruit.

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モモ‘紅清水’における落果および種子発育に及ぼす種子への傷害およびジベレリンとパクロブトラゾールの注入の影響

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モモ‘紅清水’において種子への傷害が誘起する落果と種子発育との関係を検討した．種子への傷害（傷害処理）として果実の縫合線と逆の位置から種皮まで外径2.5㎜のドリルを満開後40，50，60，72および80日に挿入した．落果や種子発育と関係するジベレリンとパクロブトラゾールの種子への注入（注入処理）も傷害処理後に行った．傷害処理，注入処理ともに，満開後40～60日処理には処理した果実が全て落下したのに対し，72および80日処理では，いずれも落下しなかった．60日処理では，果実および種子の新鮮重がともに処理３日後に減少し始めた．胚乳と胚の形態を満開後60，72および80日処理間で比較した．胚乳および胚の成長は60日処理では処理３日後に停止したが，72日処理では胚が処理５日後まで成長し続けた．胚乳と胚の細胞核の崩壊は，処理時の胚長が５㎜未満であった60日処理では，処理３日後に生じた．一方，処理時の胚長が約８㎜であった72日処理では，胚乳における細胞核の崩壊は60日処理と同様の傾向で生じたが，胚の細胞核は崩壊しなかった．60日処理において合点側吸器の形態を観察したところ，吸器は傷害処理や注入処理によって直ちに収縮し始め，正常な合点側吸器を持つ種子の割合は急速に低下した．これらの結果に基づいて，種子への傷害による種子の退化過程ならびに胚の成長と落果との関係を考察した．