The Growth Inhibitors in Sugar Beet Seed Balls

III. Changes of growth inhibitors contents in seed balls during maturity

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In the previous papers^{5,6,7)} on the growth inhibitors in sugar beet (*Beta vulgaris*) seed balls, we suggested that mono-sodium oxalate as a root growth inhibitor, potassium nitrate as a germination inhibitor and several phenolic compounds as minor inhibitors were present in the "water extract" of seed balls.

It was reported that the seeds showed the higher percentages of germination in the late maturity stage²⁾. The inhibitor content was found to decrease progressively with the increase in maturity of seed balls by BATTLE et al.³⁾

In this paper we tried to investigate the changes of the growth inhibitors contents in seed balls during maturity. And it may arise that the water soluble inhibitors are being leached off from seed balls by rainfall in the period of maturity of sugar beet.

For that reason, the experiments of the overhead irrigation treatment to simulate rainfall was undertaken.

Materials and Methods

Sugar beet variety Dônyû No. 2 used in the experiments in order to investigate the inhibitors contents in seed balls at the different harvest dates was sown in the field on June 3, 1973 and cultivated by direct seeding.

The first harvest of sugar beet seed balls was made on June 13, 1974 after 20 days from the beginning time of flowering. Subsequent harvests of seed balls were made on June 24, July 3 and 16 in intervals of 10 days.

Sugar beet variety Dônyû No. 2 used in the experiments of the overhead irrigation treatment was sown on September 3, 1974

and grown in 1/2000 a Wagner pot in which a rubber stopper was removed to drain. Sugar beets grown in pots were moved into a net house spreaded with vinyl layer above the head to exclude from rain. An artificial raindrop producer manufactured in Daiki Kôgyô Co. Ltd was used for the overhead irrigation treatment.

Sugar beets were irrigated from overhead spray nozzle for 2 minutes to simulate heavy rainfall at intervals of 22 minutes and the overhead irrigation treatment had five replicates in a day. The rainfall was 20 mm per day.

The overhead irrigation treatment was started on June 15 and continued for the following 30 days. Sugar beet seed balls were harvested on July 15.

Preparations of the "water extracts", the washing of seed balls, the seedling growth and germination tests were carried out in the same methods as described in the previous papers.^{5,6,7)}

Quantitative analyses of nitrate contents were determined according to the method described by ADRIAANSE and ROBBERS.¹⁾ Quantitative analyses of oxalic acid contents were determined according to the method described by SNYDER et al.¹³⁾

The paper chromatographies were also carried out as the following methods. Two hundred ml of the "original water extract" was evaporated and the resulting residue was taken up to 5 ml of distilled water and this 5 ml of the "concentrated extract" was applied as a band on 24—28 sheets of 40×40 cm Tôyô No. 50 filter paper and chromatographed by ascending development using ethanol—acetic acid—water (8:2:5 v/v/v) as the solvent. The paper chromato-

grams were divided into segments 0.05 Rf long and zones belonging to the same segment were collected from the chromatograms respectively and subsequently eluted with each 300 ml of distilled water.

The each resulting eluate was evaporated and the residue was dissolved in 20 ml of distilled water. The each eluate was assayed by germination of "washed seed balls". The each 4 ml eluate was diluted to 10 ml and the diluted eluate was assayed by seedling growth of sugar beet.

Seed balls used for assays of the experiments for investigation of the inhibitors contents of seed balls at the different harvest dates was variety Dônyû No. 2 harvested on July 16, 1974.

Seed ball used for assays of the experiments of the overhead irrigation treatment was variety Hon-iku No. 192 harvested on July 16, 1974.

Each assay had four replicates respectively.

Results

The percentages of germination of "unwashed seed balls" at the different harvest dates were shown in Fig. 1. In the late maturity stage seed balls showed the high percentages of germination. The percentage of germination of seed balls harvested finally was 74.7% at 7 days after seeded.

Fig. 1. The percentages of germination of "unwashed seed balls" harvested at intervals of 10 days during maturity of seed balls.

The percentage of germination of "washed seed balls" harvested on June 13 was 26.0%, but the percentages of germination of seed balls harvested subsequently were the high percentages of germination (Fig. 2).

Seed balls after 20 days from flowering of one sugar beet plant were still immature, but those after 30 days from flowering developed well and had the good germination ability.

Therefore the inhibitors contents were found to decrease gradually during maturity of seed balls.

This was further confirmed as follows. Variation in germination of seed balls at different concentrations of the "water extracts" of seed balls at the different harvest dates was shown in Fig. 3. The "water extract" of sugar beet seed balls harvested in the early maturity stage inhibited germination of "washed seed balls" considerably, but the "water extract" of sugar beet seed balls harvested finally on July 16 hardly inhibited germination of "washed seed balls".

Variation in growth of seedlings at different concentrations of the "water extracts" of sugar beet seed balls at the different harvest dates was shown in Fig. 4. The inhibitory activity of the "water extracts" showed a constant decrease with maturity of

Fig. 2. The percentages of germination of "washed seed balls" harvested at intervals of 10 days during maturity of seed balls.

Fig. 3. Effects of the "water extracts" of seed balls harvested at intervals of 10 days on germination of "washed seed balls". The percentages of germination were recorded at 4 days after seeded. Concentration was represented by ratio to the concentration of the "original water extract". The "original water extract" was prepared by shaking 100g of seed balls with 400 ml of distilled water for 3 hours at room temperature and then filtrated. Cont. indicated the test using distilled water.

seed balls.

In order to clarify the changes of the contents of mono-sodium oxalate as a root growth inhibitor, potassium nitrate as a germination inhibitor and several phenolic compounds as minor inhibitors during maturity, the paper chromatographies were carried out using the "water extracts" of seed balls at the different harvest dates. The

Fig. 4. Effects of the "water extracts" of seed balls harvested at intervals of 10 days on elongation of seedling roots during 24 hours. Concentration and cont. as in Fig. 3.

Fig. 5. Histograms of the inhibitory action of the eluates from different Rf zones of chromatograms of the "water extracts" of seed balls harvested at intervals of 10 days on germination of "washed seed balls". Broken lines indicated the germination tests using distilled water. The percentages of germination were recorded at 4 days after seeded.

Fig. 6. Histograms of the inhibitory action of the eluates from different Rf zones of chromatograms of the "water extracts" of seed balls harvested at intervals of 10 days on elongation of seedling roots during 24 hours. Broken lines indicated the seedling growth tests using distilled water.

Table 1. Oxalic acid and nitrate contents in each "original water extract" of seed balls harvested at intervals of 10 days.

	13/VI	24/VI	3/VII	16/VII
(COOH) ₂ mg/ml	0.648	0.639	0.540	0.117
NO ₃ μg/ml	708.75	301.05	128.25	83.70

bioassays by germination tests indicated that the strong inhibitory action for potassium nitrate was seen at Rf 0.55—0.60. And the inhibitory action of this zone decreased with the increase in maturity of seed balls (Fig. 5). Therefore it was clarified that

Fig. 7. The percentages of germination of "unwashed seed balls" and "washed seed balls" of sugar beet grown under the dry and wet conditions.

Fig. 8. Effects of the "water extracts" from seed balls of sugar beet grown under the dry and wet conditions on germination. The percentages of germination were recorded at 4 days after seeded. Concentration and cont. as in Fig. 3.

the decrease of the inhibitory activity of the "water extracts" with maturity attributed to the decrease in the contents of potassium nitrate.

Histograms of seedling growth tests were shown in Fig. 6. In the histograms of June 13 and 24, the inhibitory zones extended

Fig. 9. Effects of the "water extracts" from seed balls of sugar beet grown under the dry and wet conditions on elongation of seedling roots during 24 hours. Concentration and cont. as in Fig. 3.

Fig. 10. Histograms of the inhibitory action of the eluates from different Rf zones of chromatograms of the "water extracts" of seed balls of sugar beet grown under the dry and wet conditions on germination of "washed seed balls". Broken lines indicated the germination tests using distilled water. The percentages of germination were recorded at 4 days after seeded.

from Rf 0.45 to Rf 0.95. Histogram of July 3 indicated that the inhibitory action of the zone Rf 0.50—0.55, suggesting that mono-sodium oxalate located, was weak when compared with the zones Rf 0.60—0.90, suggesting that several phenolic compounds and others located. Especially the inhibitory action of the zone Rf 0.50—0.55 was very weak as compared with the zones Rf 0.60—0.90 in histogram of July 16. The decrease of the inhibitory action with maturity attributed mainly to the decrease in the contents of mono-sodium oxalate.

Also oxalic acid and nitrate contents in each "water extract" decrease with the increase in maturity of seed balls (Table 1).

Sugar beets were grown differently under the wet condition (overhead irrigation) and the dry condition (no overhead irrigation) for the purpose of the investigation of leaching off the growth inhibitors from seed balls by rainfall during maturity of seed

Fig. 11. Histograms of the inhibitory action of the eluates from different Rf zones of chromatograms of the "water extracts" of seed balls of sugar beet grown under the dry and wet conditions on elongation of seedling roots during 24 hours. Broken lines indicated the seedling growth tests using distilled water.

balls.

Seed balls from plants grown under the dry condition germinated better than those under the wet condition (Fig. 7). It was recognized that rainfall decreased the percentages of germination by delaying the onset maturity.

The difference of the percentages of germination due to the overhead irrigation treatment was considerably little in the effects of the "water extracts" of seed balls from plants grown under the wet and dry conditions (Fig. 8). The inhibitory activity of the "water extract" of seed balls from plants grown under the dry condition was slightly stronger than that under the wet condition on the root growth (Fig. 9).

The paper chromatographies were carried out using the "water extracts" in order to investigate whether the inhibitors contents in the "water extract" of seed balls from plants grown under the wet condition were different from those grown under the dry condition or not.

Histograms of the percentages of germination were shown in Fig. 10. Histograms of the wet and dry conditions showed that the zone Rf 0.55—0.60 had the strongest inhibitory action when compared with other zones. The inhibitory action of the zone Rf 0.55—0.60 of the dry condition was slightly stronger than that of the wet condition.

Histograms of seedling growth tests were shown in Fig. 11. Histograms of the wet and dry conditions showed that the zone Rf 0.50—0.55 was weak when compared with other zones as shown in July 16 in Fig. 6.

The water soluble inhibitors were not leached off from seed balls by rainfall in the period of maturity.

Discussion

Seed balls contained enough mono-sodium oxalate, potassium nitrate and several phenolic compounds to act as strong growth inhibitors in the early maturity stage.

Mono-sodium oxalate and potassium nitrate decreased remarkably with the increase in maturity of seed balls but phenolic com-

pounds were not found remarkably as did mono-sodium oxalate and potassium nitrate.

Oxalate like mono-sodium oxalate was found in many plants.⁹⁾ Oxalic acid is produced and respired in mid-dayflower (*Mesembryanthemum spectabile*) and tobacco (*Nicotiana tabacum*).¹⁰⁾ And also presence of oxalic acid oxidase was known.¹⁰⁾ Therefore it could be considered that oxalate played some physiological role in plants. Thus mono-sodium oxalate found as a growth inhibitor was not waste product of metabolism and was utilized in sugar beet. Also it was reported that water insoluble oxalate like calcium oxalate was contained in sugar beet seed balls.¹²⁾ Therefore it was also considered that water insoluble oxalate is formed from water soluble oxalate.

Nitrogen is absorbed by the plant in the nitrogen form as nitrate or as ammonia. Higher plants synthesize all the twenty-odd amino acids necessary for the formation of its protein from inorganic nitrogen. In many cases the amino acid synthesis occurs in the roots of a plant, and the amino acids are translocated to the growing shoots where they are built up to form proteins.

In other plants, however, the inorganic nitrogen is translocated directly to the leaves and synthesized there amino acids and proteins.¹⁴⁾ Nitrate plants were given as one of these examples.

HATA et al.⁴⁾ reported that nitrate content of carrot plant showed a marked decrease in leaflets and petioles during the period of growth. Also MIYAZAKI et al.¹²⁾ reported that nitrate content in tomato fruits increased in process of the maturation and reached to a maximum at the mature green stage and then decreased. These indicated that the nitrogen reductase activity was lower in the immature fruit and became higher as ripeness developed.

It could be considered that in the case of sugar beet nitrate was absorbed and assimilated in their roots, and translated to seed balls in the period of seed development and that seed balls synthesized amino acids and proteins from nitrate. Therefore nitrate in seed balls decreased with the increase in maturity.

Phenolic compounds were present in sugar beet seed balls. Those compounds decreased with the increase in maturity of seed balls.

KEFFELI⁹⁾ suggested that a peak of accumulation of phenolic compounds existed in young green leaves, while in old abscising leaves the level of those substances was sometimes lower. It could be considered that these phenolic compounds closely associated with lignin biosynthesis and decreased with the end of lignification of seed balls.

Mono-sodium oxalate and potassium nitrate were isolated from seed balls supplied from Hokkaido National Agriculture Experiment Station. On sugar beet seed balls grown in the field of Nagoya University, mono-sodium oxalate was a main inhibitor in the early maturity stage, however, in the late maturity stage mono-sodium oxalate was not a main inhibitor. The difference in inhibitors contents was thought to be associated with environmental factors during the period of maturity of sugar beet seed balls.

Summary

Mono-sodium oxalate as a root growth inhibitor, potassium nitrate as a germination inhibitor, and several phenolic compounds as minor inhibitors were present in the "water extract" of sugar beet seed balls. In this paper we tried to investigate the changes of these growth inhibitors contents in seed balls during maturity of sugar beet.

Seed balls after 20 days from flowering were immature, but those after 30 days from flowering developed well and had the good germination ability.

The germination and root growth inhibitory activity of the "water extracts" of seed balls showed a decrease with maturity.

The paper chromatographies were carried out using the "water extracts" of seed balls at the different harvest dates. The germination inhibitory action of the zone Rf 0.55—0.60, suggesting that potassium nitrate located, decreased with the increase in maturity of seed balls. The root growth inhibitory action of the zone Rf 0.50—0.55, suggesting that mono-sodium oxalate located, decreased

with the increase in maturity of seed balls. Therefore it was clarified that the decrease of the germination and root growth inhibitory activity of the "water extracts" with maturity attributed to the decrease in the contents of potassium nitrate and mono-sodium oxalate.

The water soluble inhibitors were not leached off from seed balls by rainfall in the period of maturity.

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〔和 文 摘 要〕

テンサイ種球中の生長阻害物質

第3報 テンサイ種球の登熟中における生長阻害物質の推移

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テンサイ種球中には実生の根の生長を阻害するシュウ酸一ナトリウム、発芽を阻害する硝酸カリウムおよび数種のフェノール性物質などの生長阻害物質が存在している。本論文ではテンサイ種球の登熟中においてこれらの生長阻害物質がどのように推移するかについて報告する。

1) 開花後 20 日目の種球はまだ十分な発芽能力を持っていないが、30 日目以降ではかなり発芽能力を持っていることを示した。

2) 収穫時期を異にした種球からのそれぞれの水抽出液がテンサイ洗淨種球の発芽におよぼす影響は初期のものほど発芽阻害作用は強く、開花後 50 日目のものではほとんど発芽阻害作用はみられなかった。テンサイの実生の根の生長におよぼす影響についても同様で後期になるにつれて弱くなっていった。従って種球の登熟が進むにつれて生長阻害物質が減少していくことが認められた。

3) これまで見いだされた阻害物質中いずれが変動するかを明らかにするためにペーパークロマトグラフィーが用いられた。発芽阻害物質としての硝酸カリウムが存在する Rf 0.55—0.60 の部位が全期間とも最も強い発芽阻害作用を示した。しかし登熟するにつれてその部位の発芽阻害作用は弱くなっていった。従って後期の水抽出液ほど発芽阻害作用が弱くなることは硝酸カリウムの減少によっていることが認められた。また実生の根の生長阻害物質としてのシュウ酸一ナトリウムの存在する Rf 0.50—0.55 の部位の生長阻害作用も後期になるにつれて弱くなり、開花後 50 日目のものについてはかなり弱くなった。フェノール性物質等が存在している高い Rf 値の部位の生長阻害作用は登熟とともに弱くなっているが、シュウ酸一ナトリウムほど著しくはなかった。従って後期の水抽出液ほど実生の根の生長阻害作用が弱くなることはシュウ酸一ナトリウムの減少によっていることが認められた。

4) テンサイの登熟期の降雨によって種球中の生長阻害物質が溶脱し、減少することが考えられたため、降雨処理実験が行われた。降雨によって種子の発育は若干不良になることが示されたが、人工降雨区および無降雨区から得られた種球の水抽出液がテンサイ洗淨種球およびテンサイの実生の根の生長におよぼす影響については両区の間で大きな差は認められず、阻害作用は弱かった。

5) 前項の両区から得られた種球の水抽出液を分画するためにペーパークロマトグラフィーが用いられた。ペーパークロマトグラムの各部位からの水抽出液が発芽および実生の根の生長におよぼす影響をみた場合、両区の間で差は認められなかった。すなわちテンサイ種球が登熟するにつれてシュウ酸一ナトリウムおよび硝酸カリウムが減少することは降雨によってこれらの生長阻害物質が種球から溶脱するのではないことを示した。