MATROMORFIE IN *BRASSICA OLERACEA* L.

Dit proefschrift met stellingen van Albert Hendrik Eenink, landbouwkundig ingenieur, geboren te Terborg op 26 mei 1944, is goedgekeurd door de promotor, dr. ir. J. Sneep, hoogleraar in de leer van de plantenveredeling.

Wageningen, 11 februari 1975

De Rector Magnificus van
de Landbouwhogeschool,
J.P.H. van der Want

A.H. Eenink

Matromorfie in *Brassica oleracea* L.

Proefschrift ter verkrijging van de graad van doctor in de landbouwwetenschappen, op gezag van de rector magnificus, dr. ir. J.P.H. van der Want, hoogleraar in de virologie, in het openbaar te verdedigen op woensdag 23 april 1975 des namiddags te vier uur in de aula van de Landbouwhogeschool te Wageningen.

Instituut voor de Veredeling van Tuinbouwgewassen Wageningen 1975

DIT PROEFSCHRIFT VAN A.H. EENINK OMVAT DE VOLGENDE ARTIKELEN:

EENINK, A.H., 1974a. Matromorphy in *Brassica oleracea* L. I. Terminology, parthenogenesis in Cruciferae and the formation and usability of matromorphic plants. Euphytica 23: 429–433.

EENINK, A.H., 1974b. Matromorphy in *Brassica oleracea* L. II. Differences in parthenogenetic ability and parthenogenesis inducing ability. Euphytica 23: 435–445.

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EENINK, A.H., 1974d. Matromorphy in *Brassica oleracea* L. IV. Formation of homozygous and heterozygous diploid products of gametogenesis and qualitative genetical research on matromorphic plants. Euphytica 23: 719–724.

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Stellingen

1. Hybriderassen van sla zouden een zeer positieve bijdrage aan de slateelt kunnen leveren.
2. De cultuur en fusie van protoplasten worden belangrijke hulpmiddelen bij de veredeling van cultuurgewassen.
3. Apomixis bij tuinbouwgewassen krijgt te weinig aandacht, dit is mede een gevolg van onvoldoende kennis van de genetica van dit verschijnsel.
4. Het veredelen op rassen van voedselgewassen voor een groot verspreidingsgebied is in verband met de wereldvoedselproductie ongewenst.
5. Het is onjuist het verschijnsel incompatibiliteit te betrekken bij een vergelijking van stamper-pollen relatie en waard-parasiet relatie.
6. Er is geen principieel verschil tussen uniforme en differentiële resistentie.
7. Zonder veredeling op geringere energiebehoefte zal het vrijwel onmogelijk zijn de teelt gedurende de winter van de op dit moment belangrijkste groentegewassen in Nederland te handhaven.
8. Het geslacht Eruca Mill. dient te worden ondergebracht bij het geslacht Brassica L.
9. Alternatieve landbouw op grotere schaal toegepast leidt tot hongersnood.
10. De zeer ingrijpende bezuinigingen die hebben plaatsgevonden bij het landbouwkundig onderzoek in Nederland zijn uit sociaal, economisch en milieuhygiënisch oogpunt onbegrijpelijk en onverantwoord.
11. De welvaart en het welzijn in Nederland zullen grote negatieve gevolgen ondervinden van een ver doorgevoerde nivellering.
12. Bij het bedrijven van ontspanningspolitiek met Oost-Europa laat het Westen zich te veel leiden door motieven van nationaal en internationaal economische aard.
13. De etikettering van levensmiddelen m.b.t. de aanwezigheid en aard van additieven dient zo spoedig mogelijk wettelijk te worden verplicht.

Proefschrift van A.H. Eenink

Wageningen, 23 april 1975

CURRICULUM VITAE

De promovendus werd op 26 mei 1944 geboren te Terborg (Gld). Hij bezocht de HBS aan het Gemeente Lyceum te Doetinchem en begon zijn studie aan de Landbouwhogeschool te Wageningen in 1962. In 1969 werd het ingenieursdiploma met lof behaald in de richting plantenveredeling met als verdere specialisaties erfelijkheidsleer, algemene plantenziektenkunde en tropische landbouwplantenteelt. Van 1969 tot 1972 was de promovendus gedetacheerd bij het Instituut voor de Veredeling van Tuinbouwgewassen te Wageningen waar hij in het kader van een promotie-assistentschap aan de Landbouwhogeschool onderzoek verrichtte aan matromorfie in Brassica oleracea. Sinds 1972 is hij op het bovengenoemde instituut werkzaam als hoofd van de afdeling Blad- en Stengelgewassen waar door hem veredelingsonderzoek wordt uitgevoerd dat betrekking heeft op o.a. soortkruisingen, incompatibiliteit, polyploidie, weinig energie vragende teelten en resistenties tegen schimmels en insecten bij Lactuca, Cichorium en Spinacia.

MATROMORFIE IN *BRASSICA OLERACEA* L.

INLEIDING

De ontwikkeling van een diploid embryo uit een onbevruchte eicel van een diploide plant, mede ten gevolge van de stimulerende invloed van soort- of geslachtsvreemd pollen, wordt matromorfie of pseudogame diploide parthenogenese genoemd (EENINK, 1974a).

Afwijkingen in kern- of celdelingen, die resulteren in eicellen waaruit (diploide) matromorfe embryos ontstaan, kunnen vóór, tijdens, of na de meiose optreden. Het tijdstip waarop deze afwijkingen optreden beïnvloedt het homozygotieniveau van de matromorfe planten. Volgens sommige onderzoekers (TOKUMASU, 1965; MACKAY, 1972) vinden deze afwijkingen bij *Brassica* vóór of tijdens de meiose plaats, volgens anderen (NISHI et al., 1964; RÖBBELEN, 1965) na de meiose.

Wanneer matromorfe planten van *Brassica oleracea* volledig of vrijwel volledig homozygoot zouden zijn, dan zouden deze kunnen fungeren als ouders bij de produktie van F1-hybriden. Het is dan niet meer noodzakelijk, via een vaak langdurige procedure van herhaalde zelfbevruchting, inteeltlijnen te maken. Naast het homozygotieniveau van de matromorfe planten is ook het aantal beschikbare planten van belang omdat binnen een groep matromorfe planten moet worden geselecteerd op vitaliteit, de afwezigheid van ongewenste eigenschappen en het bezitten van een goede algemene en specifieke combinatiegeschiktheid.

Teneinde de mogelijkheden van het gebruik van matromorfe planten bij de produktie van F1-hybriden in *Brassica oleracea* te onderzoeken werden in dit gewas de volgende aspecten van het verschijnsel matromorfie bestudeerd.

a. De invloed van genetische verschillen op de frequentie van matromorfie.
b. De invloed van uitwendige omstandigheden op de frequentie van matromorfie.
c. Het homozygotieniveau van matromorfe planten voor kwalitatieve en kwantitatieve eigenschappen in samenhang met het tijdstip van optreden van afwijkingen in kern- of celdelingen resulterend in eicellen waaruit diploide matromorfe embryos ontstaan.
d. De microsporogenese en de groei en ontwikkeling van embryos, endospermen en zaadknoppen van prikkelbestoven *B. oleracea* planten in verband met de afwijkingen in kern- of celdelingen genoemd onder c.

HET GENETISCH MILIEU EN HET OPTREDEN VAN MATROMORFIE

Uit vroeger onderzoek is gebleken dat bij Cruciferen tussen de verschillende oudercombinaties verschillen optreden in frequentie van matromorfie (NOGUCHI, 1928; TERASAWA, 1928, 1932; OLSSON, 1960; NISHI et al., 1964; PRAKASH, 1973). Deze verschillen worden mogelijk veroorzaakt door zowel verschillen in parthenogenetisch vermogen (p.v.) als door verschillen in parthenogenese inducerend vermogen (p.i.v.). Om bij een eventuele homozygotie van de matromorfe *Brassica oleracea* planten over grote aantallen van deze planten te kunnen beschikken werd bij Cruciferen onderzoek uitgevoerd naar verschillen in p.v. en p.i.v. tussen soorten, variëteiten, rassen en planten per ras.

Planten van 13 rassen van *Brassica oleracea,* behorend tot de variëteiten *capitata, gemmifera, gongylodes, laciniata* en *sabauda* werden bij 14°C en 17°C prikkelbesto-

ven door vertegenwoordigers van *Brassica rapa, B.japonica, B.nipposinica, Camelina sativa, Crambe abyssinica, Eruca sativa* en *Raphanus sativus*. Het bleek dat er tussen populaties van verschillende rassen van één variëteit significante verschillen optraden in p.v. en in p.i.v. Sommige combinaties produceerden relatief zeer veel matromorfe (en/of hybride) zaden. De hoeveelheid gevormde matromorfe zaden was in absolute zin in de meeste gevallen echter gering.

Met behulp van klonen, gemaakt van planten uit populaties van *B.oleracea* rassen, werden verschillen in *i*ndividueel *p*arthenogenetisch *v*ermogen (i.p.v.) onderzocht. Sommige klonen bleken relatief veel, andere bleken geen matromorfe zaden te produceren (EENINK, 1974b). Tussen verschillende planten van een populatie van een prikkelbestuiver (*Eruca sativa*) werden aanzienlijke verschillen in *i*ndividueel *p*arthenogenese *i*nducerend *v*ermogen (i.p.i.v.) aangetroffen. Zeer waarschijnlijk wordt een deel van de verschillen in p.v. of p.i.v. tussen soorten, variëteiten, rassen of planten, genetisch bepaald.

Resultaten van onderzoek naar de genetica van een groot parthenogenetisch vermogen duiden op een niet-recessieve overerving.

TEMPERATUUR, VERLATE PRIKKELBESTUIVING EN TOEDIENING VAN GROEIREGULATOREN EN HET OPTREDEN VAN MATROMORFIE

Om de invloed van uitwendige omstandigheden op het optreden van matromorfie te onderzoeken werd gebruik gemaakt van klonen van de *Brassica oleracea variëteiten capitata, gemmifera* en *sabauda*. Iedere kloon werd prikkelbestoven door één plant van een ander geslacht of een andere soort.

Planten van deze klonen werden gedurende de pre-meiose en de meiose bij drie temperaturen (10, 14 en 17°C) geplaatst, waarna prikkelbestuiving en zaadzetting bij één temperatuur (14°C) plaatsvond. Uit dit onderzoek bleek dat in sommige gevallen de temperatuur, voor of tijdens de meiose, invloed uitoefende op het aantal gevormde matromorfe zaden. Dit impliceert dat mogelijk afwijkingen in kern- of celdelingen voor of tijdens de meiose, de frequentie van de diploide parthenogenese kunnen beïnvloeden.

Soortgelijk onderzoek naar de invloed van de temperatuur tijdens en na de prikkelbestuiving op het optreden van matromorfie toonde aan dat matromorfe zaden bij de diverse temperaturen in verschillende frequenties werden gevormd. Dit zou kunnen wijzen op chromosoomverdubbeling van de eicel na prikkelbestuiving. Het is echter ook mogelijk dat het meer of minder optimaal zijn van de temperatuur voor het uitgroeien van eicellen, die in een vroeger stadium reeds diploid zijn geworden, een rol speelt.

Wanneer prikkelbestuiving 48 of meer uren na emasculatie werd uitgevoerd, dan bleken er significant meer matromorfe (en ook meer hybride) zaden te worden gevormd dan wanneer prikkelbestuiving onmiddellijk volgde op de emasculatie. Met behulp van microscopisch onderzoek (UV-methode) werd aangetoond dat de toegenomen zaadzetting zeer waarschijnlijk een gevolg was van het binnendringen van grotere aantallen pollenbuizen in de stampers.

Na toediening van groeiregulatoren (GA3, NAA en Ethrel) aan prikkelbestoven bloemknoppen bleek dat GA3-oplossingen een significant positieve invloed uitoefenden op het aantal matromorfe zaden dat werd gevormd (EENINK, 1974c).

ONDERZOEK AAN KWALITATIEF EN KWANTITATIEF GENETISCHE EIGENSCHAPPEN VAN MATROMORFE PLANTEN

Teneinde de mate van homozygotie en de ontstaanswijze van de matromorfe embryo's te onderzoeken werden de genotypen geanalyseerd van kwalitatieve eigenschappen (incompatibiliteit, glanzend blad, anthocyaan, bladkroezing) van matromorfe planten die waren verkregen na prikkelbestuiving van planten met een heterozygoot genotype voor één of meer van deze eigenschappen. Uit de aanwezigheid van matromorfe planten met heterozygote genotypen voor de zojuist genoemde eigenschappen werd afgeleid dat althans een deel van de matromorfe planten niet diploid was ten gevolge van afwijkingen in kern- of celdelingen na de meiose. Het was niet mogelijk met behulp van de genotypenfrequenties voor de verschillende eigenschappen, uitspraken te doen t.a.v. het moment waarop chromosoomverdubbeling had plaatsgevonden omdat de plaats op het chromosoom van de betreffende loci niet bekend was en bovendien verschillende afwijkingen in kern- of celdelingen mogelijk waren (EENINK, 1974d).

Zowel vrij slecht als goed groeiende matromorfe planten werden na prikkelbestuiving van planten van open bestoven en hybride rassen verkregen. Na vergelijken van gemiddelden en varianties voor kwantitatieve eigenschappen (stengellengte, koolgewicht) van populaties matromorfe planten en controle planten (I1's en uitgangspopulaties), bleek dat de matromorfe planten niet in een inteeltminimum verkeerden. Na zelfbevruchting van matromorfe planten vertoonden de nakomelingen inteeltdepressie. Van planten uit populaties verkregen na diallele kruisingen tussen matromorfe planten en tussen controle planten (I1's) werden de stengellengtes gemeten en de varianties bepaald waarna variantieanalyses werden uitgevoerd. In een aantal gevallen bleken zowel het algemeen als het specifiek combinatievermogen een belangrijke invloed uit te oefenen op de stengellengte; de stengellengte werd hierbij dus zowel door additieve als niet-additieve genwerking bepaald. Uit de grote verschillen tussen de diverse populaties in variantie voor stengellengte kon worden afgeleid dat er tussen de matromorfe planten aanzienlijke verschillen in homozygotieniveau van het genotype voor stengellengte bestonden. Deze verschillen waren waarschijnlijk een gevolg van diverse verstoringen in kern- of celdelingen voor of tijdens de meiose. Sommige planten waren dermate heterozygoot dat de conclusie getrokken werd dat ze waarschijnlijk waren ontwikkeld uit diploide eicellen met hetzelfde genotype als de heterozygote (hybride) moederplant. Andere planten leken vrij homozygoot, deze waren misschien ontwikkeld uit eicellen die diploid waren ten gevolge van afwijkingen tijdens de tweede reductiedeling. Volledig homozygote matromorfe planten werden niet aangetroffen (EENINK, 1974e).

ZAADKNOPPEN, EMBRYOS EN ENDOSPERMEN

Onderzoek aan zaadknoppen, embryos en endospermen van prikkelbestoven *B.oleracea* planten werd uitgevoerd om meer informatie te verkrijgen over a. het tijdstip van chromosoomverdubbeling resulterend in diploide eicellen en b. de ontwikkeling en groei van deze zaadknoppen, embryos en endospermen.

Bestudering van microtoompreparaten toonde aan dat zaadknoppen langzamer groeiden na prikkelbestuiving dan na zelfbestuiving. Ook de embryogroei en -ontwikkeling en de endospermgroei verliepen na prikkelbestuiving langzamer dan na zelfbestuiving. De geringe embryoafmetingen correspondeerden met een tragere

groei en ontwikkeling van de matromorfe planten (gedurende de eerste 40 dagen na zaaien) vergeleken met de ontwikkeling van corresponderende I1 planten. De trage groei en ontwikkeling van embryos en endospermen werden waarschijnlijk veroorzaakt door kwalitatieve en/of kwantitatieve genoomverschillen binnen de embryozak (RUTISHAUSER, 1969). In sommige embryozakken trad waarschijnlijk een autonome endospermontwikkeling op. Ondanks de afwezigheid van generatieve kernen werden namelijk gefuseerde poolkernen aangetroffen.

Uit de diameters van de endospermkernen werd geconcludeerd dat sommige embryozakken diploid waren, hetgeen eveneens wijst op een chromosoomverdubbeling van de eicel t.g.v. afwijkingen in kern- of celdelingen vóór of tijdens de meiose (EENINK, 1975a).

MICROSPOROGENESE EN MICROSPOROGENESE-PRODUKTEN

Eicellen waaruit matromorfe embryos ontstaan, zijn waarschijnlijk diploid ten gevolge van afwijkingen in kern- of celdelingen voor of tijdens de meiose. Onderzoek van de microsporogenese toonde aan dat er storingen optraden bij de vorming van spoelfiguren en bij de verdeling van de chromosomen over de anafase II produkten. Afwijkende microsporogenese-produkten zoals ongereduceerde PMC's, dyaden en triaden werden aangetroffen. De afwijkende microsporogenese-produkten correspondeerden met reuzepollenkorrels die door dezelfde planten werden geproduceerd. Een deel van de reuzepollenkorrels bezat twee (waarschijnlijk diploide) generatieve kernen, een deel bezat 4 (waarschijnlijk haploide) generatieve kernen. Hieruit kon worden afgeleid dat cytokinese en karyokinese zich in zekere mate onafhankelijk van elkaar gedroegen. Er werd een significante positieve correlatie aangetroffen tussen de frequentie van de reuzepollenkorrels en de frequentie van de matromorfe zaden bij een aantal klonen van een ras van *Brassica oleracea*. Dit duidt erop dat de matromorfe embryos zijn ontstaan uit eicellen die diploid waren t.g.v. afwijkingen tijdens de meiose. Deze eicellen waren dus heterozygoot (EENINK, 1975b).

CONCLUSIES

De frequentie waarin matromorfie bij planten van *Brassica oleracea* optreedt blijkt zowel door het inwendig (genetisch) milieu als door het uitwendig milieu (temperatuur, verlate prikkelbestuiving, groeiregulatoren) te worden beïnvloed. De relatieve verschillen in frequentie waren in een aantal gevallen aanzienlijk (bijv. na behandeling met GA3-oplossingen en na verlate prikkelbestuiving), in absolute zin echter waren de verschillen in aantal gevormde matromorfe zaden meestal gering. Dit beïnvloedt de bruikbaarheid van de matromorfie in *Brassica oleracea* in ongunstige zin omdat de aantallen matromorfe planten die uit prikkel te bestuiven populaties worden verkregen waarschijnlijk zo gering zullen zijn dat selectie bij deze planten op o.a. goede algemene en specifieke combinatiegeschiktheid daardoor aanzienlijk wordt bemoeilijkt of onmogelijk is. Onderzoek aan kwalitatieve en kwantitatieve eigenschappen van matromorfe planten toonde aan dat de matromorfe planten heterozygoot zijn. Tussen de planten waren echter aanzienlijke verschillen in homozygotieniveau aanwezig. Sommige matromorfe planten leken een enigszins hoger niveau van homozygotie te hebben dan corresponderende controle planten ontstaan na zelfbestuiving. Volledig of vrijwel volledig homozygote matromorfe planten werden niet aangetroffen. Dit heeft tot gevolg dat de matromorfe planten niet kunnen

worden gebruikt als ouders bij de productie van F1-hybriden. Door cytologisch en embryologisch onderzoek werd aangetoond dat de verschillen in homozygotieniveau zeer waarschijnlijk een gevolg zijn van verschillende afwijkingen in kern- of celdelingen tijdens de meiose waardoor diploide heterozygote eicellen worden gevormd.

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MATROMORPHY IN *BRASSICA OLERACEA* L. I. TERMINOLOGY, PARTHENOGENESIS IN CRUCIFERAE AND THE FORMATION AND USABILITY OF MATROMORPHIC PLANTS

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SUMMARY

A review is given of literature on matromorphy in *Brassica*. Some possibilities of how the matromorphics may originate and of the consequences for the usability of the matromorphics are discussed.

INTRODUCTION

It may be attractive to make use of the matromorphy phenomenon in *Brassica oleracea* L., for if matromorphic plants are homozygous they can function as parents of hybrid varieties. However, there are many uncertainties concerning the formation and consequently the usability of the matromorphic plants. This usability is also determined by the (highly variable) frequency of matromorphic seeds in different parental combinations. Therefore a study of matromorphy in *B. oleracea* was undertaken.

TERMINOLOGY

The development of an embryo from an unfertilized nucleus or cell, resulting from the stimulating influence of for instance pollen from other species, is called pseudogamous apomixis. When a haploid or diploid embryo develops from an unfertilized egg-cell, this is refferred to as haploid respectively diploid parthenogenesis (GUSTAFSSON, 1935; RUTISHAUSER, 1967).

Parthenogenesis in Cruciferae often occurs after interspecific or intergeneric pollination. This pollination for induction of parthenogenesis is called prickle pollination, though sometimes hybrids may develop. Diploid parthenogenesis is called matromorphy; the plants are called matromorphs, matromorphics or matromorphic plants. Matromorphy should not be confused with matrocliny (KERNER, 1881; KUHN, 1930). Matroclinous plants are hybrids, (formed after fertilization of the egg-cell) which resemble the mother much more closely than the father. This may be caused by a dominant gene action, or dose effects caused by different numbers of genomes from the mother and the father in the hybrid individual (HUZIWARA, 1965). Besides matromorphy (diploid parthenogenesis) and matrocliny, patromorphy (diploid androgenesis) (DE VRIES, 1911; ABDALLA & HERMSEN, 1971) and patrocliny may occur. Patroclinous plants are hybrids which resemble the father much more closely than the mother.

HAPLOID AND DIPLOID PARTHENOGENESIS

Haploid *Brassica* plants were found by OLSSON & HAGBERG (1955). Presumably as early as 1924 KAKIZAKI found matromorphy in crosses between *B. campestris* L. and *B. oleracea* L. Both species were used as male and female parent. NOGUCHI (1928) also found matromorphics after crossing these species. Various other research workers (U, 1928; OLSSON, 1954, 1960; OLSSON & HAGBERG, 1955; OLSSON et al., 1955; HÅKANSSON, 1956; HOFFMANN & PETERS, 1958; RÖBBELEN, 1965; MACKAY, 1968, 1972; ANONYMOUS, 1969; HODGKIN & REDFERN, 1971) also obtained matromorphics after crossing different botanic varieties of the above mentioned species. The frequencies of matromorphic seeds differed per botanic variety. Other interspecific crosses, too, such as between *B. carinata* A. BRAUN, *B. juncea* CZERN., *B. nigra* KOCH., *B. pekinensis* RUPR. and *B. tournefortii* GOUAN, resulted in the development of matromorphic seeds. Sometimes *B. oleracea* or *B. campestris* were used in crosses with the above mentioned species as the female or male parent (TERASAWA, 1928, 1932; MOHAMMAD & SIKKA, 1940; RAMANUJAM, 1940, 1943; NISHI et al., 1959, 1962, 1964; HONMA & HEECKT, 1960; NARAIN & PRAKASH, 1972; PRAKASH, 1973). Matromorphic seeds were further obtained from crosses between *Brassica* species and other genera of the Cruciferae like *Crambe abyssinica* HOCHST, *Eruca sativa* MILL., *Raphanus sativus* L. and *Sinapis arvensis* L. (NISHI et al., 1959, 1962, 1964; NAKAGAWA et al., 1962., TOKUMASU, 1965, 1970).

Except in Cruciferae, parthenogenesis occurs in a great number of other plant families (RUTISHAUSER, 1967; HORN, 1972). Parthenogenesis for instance resulted in the development of monoploids in maize (CHASE, 1952a, 1952b, 1969). Crosses between *Petunia* and other genera of Solanaceae were also accompanied by the occurrence of haploid parthenogenesis (KATAYAMA & ADACHI, 1969). After interspecific crossing in *Solanum*, besides haploid parthenogenesis, diploid parthenogenesis occurs (ABDALLA & HERMSEN, 1971). Also after crosses between parents with different ploidy levels parthenogenetically formed seeds developed, e.g. in *Cyclamen persicum* (LEGRO, 1959), *Primula malacoides* (SKIEBE, 1966) and *Pelargonium zonale* (BADR & HORN, 1971). Parthenogenesis also occurs in animals (SARVELLA, 1970).

THE FORMATION AND USABILITY OF MATROMORPHIC PLANTS OF BRASSICA

Between 1920 and 1960 interspecific and intergeneric crosses in which *Brassica* was involved were made mainly to obtain hybrids for transferring characters from one parent to another and for enlarging the genetic variability of varieties or selections. Little interest was shown in the matromorphy phenomenon. After 1960 more attention was paid to this phenomenon, thanks to the increasing interest in the development of hybrid varieties in *B. oleracea*. The lengthy process of inbreeding for the production of homozygous parent lines would be unnecessary in case of homozygosity of matromorphic plants. Moreover in a population of homozygous matromorphic plants, characters governed by a number of recessive genes, would appear more frequently and more rapidly than after repeated self-fertilization of inbred lines.

Opinions differ widely as to whether the matromorphic plants are homozygous or not. The different hypotheses on the formation of matromorphics vary between the

development out of a reduced diploid egg-cell from a tetraploid EMC of a diploid plant (TOKUMASU, 1965) and the development out of a duplicated haploid egg-cell after prickle pollination of the diploid female parent. After embryological and cytological research of ovules, embryos and endosperms developed after prickle pollination, or after qualitative genetical research of the matromorphic plants themselves, some research workers concluded that matromorphic plants were completely homozygous (NOGUCHI, 1928; NISHI et al., 1964; RÖBBELEN, 1965). However, other research workers showed, also by qualitative genetical research, that at least a number of the matromorphic plants were heterozygous (TOKUMASU, 1965, 1970; HODGKIN & REDFERN, 1971; MACKAY, 1972). Progeny of matromorphic plants obtained by selfing, showed inbreeding depression (NISHI, et al., 1964).

Besides the level of homozygosity of the matromorphic plants, the frequency of matromorphic plants from a given parental combination is of great importance. For within the populations of matromorphics, plants should be selected that show no unfavourable characters and have good general and good specific combining abilities with other matromorphic plants. Between the different crossing combinations there are in fact very considerable differences in frequencies of matromorphic seeds formed. Some parental combinations produce few or no matromorphic seeds at all (NISHI et al., 1964); in other combinations, however, matromorphic seeds are formed in high frequencies (PRAKASH, 1973). The environment also seems to influence the number of matromorphic seeds formed after prickle pollination (RÖBBELEN, 1965).

SCOPE OF RESEARCH

Because of the diverging opinions as to how matromorphic embryos develop, a research on the matromorphy phenomenon in *Brassica oleracea* L. was started in 1969. The following investigations have been carried out.

1. Study of the influence of different male and female parents on the frequency of matromorphic seeds produced.
2. Study of the influence of temperature, application of growth regulators and delayed prickle pollination on the number of matromorphic seeds produced.
3. Qualitative genetical research on matromorphic plants and comparison of averages and variances for quantitative characters in matromorphic and control plants and in their progeny.
4. Embryological research on ovules (embryos and endosperms) after prickle pollination and cytological research on deviating products of sporogenesis and gametogenesis in prickle pollinated mother plants.

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MATROMORPHY IN *BRASSICA OLERACEA* L. II. DIFFERENCES IN PARTHENOGENETIC ABILITY AND PARTHENOGENESIS INDUCING ABILITY

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SUMMARY

A terminology is proposed for parthenogenetic ability (p.a.) and parthenogenesis inducing ability (p.i.a.), depending on different parental combinations. An analysis of the results of many prickle pollinations shows that there were large differences in parthenogenetic ability and parthenogenesis inducing ability between genera, species, botanic varieties, varieties or accessions and plants or clones.

INTRODUCTION

Matromorphic plants can only be used as parents for hybrid varieties if they are completely or almost completely homozygous (MACKAY, 1968). It is of great importance, too, that a large number of matromorphic plants should be available. Also in connection with research on the formation of matromorphic embryos and the influence by extraneous factors on the frequency of matromorphic seeds formed after prickle pollination, parental combinations producing matromorphic seeds in a high frequency are important.

Several research workers have shown that in Cruciferae between different parental combinations great variations occur in the frequency of matromorphic seeds formed (KAKIZAKI, 1924; NOGUCHI, 1928; TERASAWA, 1928, 1932; MOHAMMAD & SIKKA, 1940; OLSSON, 1960; NAKAGAWA et al., 1962; NISHI et al., 1964; RÖBBELEN, 1965; NARAIN & PRAKASH, 1972; PRAKASH, 1973).

Reciprocal crosses may sometimes also result in greatly varying frequencies of matromorphic seeds (HÅKANSSON, 1956; HOFFMANN & PETERS, 1958; MOHAMMAD & SIKKA, 1940; RÖBBELEN, 1965). It is very likely that this variation is caused both by a difference in parthenogenetic ability and by a difference in parthenogenesis inducing ability (MOHAMMAD & SIKKA, 1940). These differences are partly genetically determined, though external circumstances may also play an important role.

This article surveys the results of research into differences in parthenogenetic ability and parthenogenesis inducing ability.

PROPOSED TERMINOLOGY FOR PARTHENOGENETIC ABILITY (P.A.) AND PARTHENOGENESIS INDUCING ABILITY (P.I.A.)

In this review a terminology is proposed for parthenogenetic ability (p.a.) and parthenogenesis inducing ability (p.i.a.) in different parental combinations. In all cases the criterium for p.a. and p.i.a. is the number of matromorphic seeds formed per 100

Table 1. Terminology for parthenogenetic ability and parthenogenesis inducing ability in different parental combinations.

Parental combination		Terminology	
female parent	male parent	parthenogenetic ability	parthenogenesis inducing ability
population	population	p.p.a.(b)	p.p.i.a.(b)
population	individual	p.p.a.(n)	i.p.i.a.(b)
individual	population	i.p.a.(b)	p.p.i.a.(n)
individual	individual	i.p.a.(n)	i.p.i.a.(n)

prickle pollinated flower buds. The terminology used is presented in Table 1 and further explained in the text.

Explanation of the terminology presented in Table 1:

Parent population = different plants from a population of a family, genus, species, botanic variety, or accession.

Parent individual = a plant or clone.

Dependent on the nature of the parents the parthenogenetic ability is indicated as follows;

p.p.a.(b) = populational parthenogenetic ability in a broad sense

p.p.a.(n) = populational parthenogenetic ability in a narrow sense

i.p.a.(b) = individual parthenogenetic ability in a broad sense

i.p.a.(n) = individual parthenogenetic ability in a narrow sense

Dependent on the nature of the parents the parthenogenesis inducing ability is indicated as follows;

p.p.i.a.(b) = populational parthenogenesis inducing ability in a broad sense

i.p.i.a.(b) = individual parthenogenesis inducing ability in a broad sense

p.p.i.a.(n) = populational parthenogenesis inducing ability in a narrow sense

i.p.i.a.(n) = individual parthenogenesis inducing ability in a narrow sense

In order to distinguish the p.p.a.(b) and p.p.i.a.(b) for female and male parents from the p.p.a.(b) and p.p.i.a.(b) of specific parental combinations, respectively, the terms general and specific p.p.a.(b) and p.p.i.a.(b) are introduced.

METHODS AND MATERIAL

In interspecific and intergeneric crossing the flowering of the parents was synchronized as much as possible by manipulating sowing dates and temperatures for the male parents. Prickle pollinations of buds from *Brassica oleracea* L. plants were done one or two days before flowering; the flower buds were emasculated with a pair of scissors, disinfected with alcohol 96%. Emasculation was immediately followed by prickle pollination. The pollinated buds of an inflorescence were enclosed in pergamyn bags; about three weeks after prickle pollination these were removed and the siliques harvested about three or four months later. Research on differences in p.p.a.(b) and p.p.i.a.(b) was done under controlled conditions in a phytotron at constant temperatures of both 14 and 17°C, at a daylength of 16 hours and a relative humidity of about

80%. Research on differences in i.p.a.(n) and i.p.i.a.(n) was done under the same conditions at 14°C. The seed set per parental combination was expressed as the number of seeds formed per 100 prickle pollinated flower buds. The seeds were sown and afterwards the matromorphic and hybrid plants were recognized by their habitus. Many seeds did not germinate. From results of research to be published later it appeared that most of these seeds contained no or very small, sometimes not yet fully developed, embryos. Some of these seeds germinated on nutrient medium, they all appeared to be hybrids.

The research into differences in p.p.a.(b) and p.p.i.a.(b) was done with the female and male parents mentioned in Table 2. Not all 13 (female parents) × 15 (male parents) possible crosses could be made owing to lack of female or male parent plants or uneven flowering of these plants. About 200 buds of different plants were pollinated per parental combination per temperature.

Twelve clones each of ten plants were made from twelve plants from a population of *Brassica oleracea* L. var. *capitata* L. cv. Kolos. The plants/clones may differ in i.p.a.(n), because Kolos is an open pollinated variety. All 12 × 10 plants were prickle pollinated by one plant of *Raphanus sativus* L. var. *radicula* PERS. cv. Huizer's Triplo.

Six genetically different plants from a population of *R. sativus* L. var. *radicula* PERS. cv. Huizer's Triplo were used as prickle pollinators for 6 × 6 plants of a clone of *B. oleracea* L. var. *capitata* L. cv. Kolos. From a population of *Eruca sativa* MILL. accession 69039, six genetically different plants were used as prickle pollinators for 6 × 5 plants of another clone of the variety Kolos, and six other *E. sativa* plants from the same population as prickle pollinators for 6 × 8 plants of a clone of *B. oleracea* L. var. *gemmifera* (DC.) SCHULZ cv. Hybride 69002.

RESULTS AND DISCUSSION

Differences in p.p.a.(b) and p.p.i.a.(b)

Table 3 gives the numbers of matromorphic and hybrid seeds formed per 100 prickle pollinated flower buds in different parental combinations. These figures are averages of the frequencies of seeds formed per combination in two experiments at 14 and 17°C, respectively. The species and botanic varieties in Table 3 are arranged in decreasing order of the frequency of seeds formed per parent. The diploid varieties of *B. rapa* var. *rapa* on the one hand and the tetraploid variety Novotas on the other hand are mentioned separately in Table 3.

Table 3 shows that some parental combinations were not made. Some combinations produce seeds in high frequencies in contrast with others, many combinations produce no seeds at all. Absence of some combinations, besides the large variation per parent in frequency of seeds formed in different combinations, causes problems in estimating differences in p.p.a.(b) and p.p.i.a.(b) between species, botanic varieties, and selections varieties or accessions. Therefore an analysis of variance on these quantitative data is not allowed unconditionally. In order to analyse differences between female or male parents, analyses of variance were made on values 1 and 0, assigned to combinations which respectively produced or did not produce seeds for the characters, numbers of matromorphic seeds (m) and numbers of hybrid seeds (h). The means for female or male parents obtained from these values were made comparable by correction for

Table 2. Female parents (2n = 18) and male parents used in crosses carried out for research into differences in p.p.a. and p.p.i.a.

Female parents		Male parents		
botanic variety of *Brassica oleracea* L.	variety or selection	species and botanic variety	variety, selection or accession	number of chromosomes
capitata L.	Kolos	*Brassica rapa* L. var. *rapa*	Ponda	2n = 20
capitata L.	Langedijker Vroege Herfst	*Brassica rapa* L. var. *rapa*	Civasto	2n = 20
capitata L.	Roem van Enkhuizen	*Brassica rapa* L. var. *rapa*	Jobe	2n = 20
gemmifera (DC.) SCHULZ	Huizer	*Brassica rapa* L. var. *rapa*	Novotas	2n = 40
gemmifera (DC.) SCHULZ	Hybride 69002	*Brassica japonica* SIEB	69004	2n = 20
gemmifera (DC.) SCHULZ	Rubine	*Brassica nipposinica* L. H. BAILEY	69012	2n = 20
gongylodes L.	Goliath	*Camelina sativa* (L.) CRANTZ	69050	2n = –
gongylodes L.	Primavera	*Crambe abyssinica* HOCHST.	69042	2n = 30
laciniata (L.) SCHULZ	Dwarf Blue Scotch	*Crambe abyssinica* HOCHST.	69043	2n = 30
laciniata (L.) SCHULZ	Roem van Hees	*Eruca sativa* MILL.	69022	2n = 22
laciniata (L.) SCHULZ	Westlandse Herfst	*Eruca sativa* MILL.	69039	2n = 22
sabauda (L.) SCHULZ	Hammer	*Raphanus sativus* L. var. *oleiformis* PERS.	Ranola	2n = 18
sabauda (L.) SCHULZ	Groene putjes	*Raphanus sativus* L. var. *oleiformis* PERS.	Siletta's	2n = 18
		Raphanus sativus L. var. *radicula* PERS.	Huizer Triplo	2n = 18
		Raphanus sativus L. var. *radicula* PERS.	Ronde Helderr.	2n = 18

missing combinations. The means multiplied by 100, resulted in the percentages of successful combinations per female or male parent as indicated in the last rows and columns for m and h in Table 3. Table 4 shows the results of the analyses of variance.

Differences in general p.p.a.(b) and p.p.i.a.(b)
Differences in p.p.a.(b) between botanic varieties. Table 3 illustrates that there are considerable differences in p.p.a.(b) between the botanic varieties investigated. Var. *gemmifera* and var. *sabauda* form significantly more matromorphic seeds than for instance var. *gongylodes*. Between botanic varieties there are also considerable differences in frequencies of hybrid seeds formed after prickle pollination.

Differences in p.p.a.(b) between varieties. From the F-value in Table 4 it appears that between varieties which belong to one or different botanic varieties significant differences occur in p.p.a.(b). Within the var. *gemmifera*, the variety Hybride 69002 differs significantly in p.p.a.(b) from the varieties Rubine and Huizer. Within the var. *capitata* there are also rather large differences in p.p.a.(b) between the varieties investigated; in the var. *gongylodes* and, to a lesser extent, in the var. *laciniata* these differences, however, are small. The varieties Hammer, Groene Putjes and Kolos also have rather great values for p.p.a.(b), in contrast to the varieties Dwarf Blue Scotch, Westlandse Herfst, Roem van Enkhuizen, Langedijker Vroege Herfst, Goliath and Primavera with very low p.p.a.(b)'s. Hybrids are formed by the different varieties in varying frequencies.

Differences in p.p.i.a.(b) between species or botanic varieties. Table 3 illustrates that, after prickle pollination with different species or botanic varieties, there are large differences in p.p.i.a.(b).

After prickle pollination with *Eruca sativa* or *Raphanus sativus* var. *oleiformis* matromorphic seeds are formed in high frequencies. P.p.i.a.'s of these prickle pollinators differ significantly from those of *B. nipposinica*, *B. japonica*, *Camelina sativa* and *Crambe abyssinica*. After prickle pollination with different species or botanic varieties the frequencies of hybrid seeds also vary greatly.

Differences in p.p.i.a.(b) between varieties or accessions. From the F-value in Table 4 it appears that there are significant differences in p.p.i.a.(b) after prickle pollination with different varieties or accessions of species. After prickle pollination with *B. rapa* var. *rapa* cv. Jobe, significantly more matromorphic seeds are formed than after prickle pollination with the variety Civasto of the same botanic variety, or after prickle pollination with varieties or accessions of other botanic varieties or species. Sometimes the differences in p.p.i.a.(b) within a species or botanic variety between accessions or varieties are small, as for instance in *E. sativa*, in *R. sativus* var. *radicula* and *oleiformis*, and in *C. abyssinica*. The varieties Siletta and Ranola, and accessions 69022 and 69039 of *E. sativa* also have a rather large p.p.i.a.(b). Various other accessions of species or varieties, like 69042 and 69043 of *C. abyssinica* and the variety Novotas, have a small p.p.i.a.(b). After prickle pollination with different varieties or accessions varying frequencies of hybrid seeds are formed.

Table 3. Numbers of seeds formed per 100 prickle pollinated flower buds in different parental combinations. m = matromorphic seeds; h = hybrid seeds.

Female parent	Male parent	*Raphanus sativus* var. *oleiformis*		*Eruca sativa*				*Brassica rapa* var. *rapa*					
		Siletta		Ranola		69022		69039		Jobe		Ponda		Civasto	
		m	h	m	h	m	h	m	h	m	h	m	h	m	h
Brassica oleracea var. *gemmifera*	Hybride 69002	0	0.5	0.2	0	1.2	2.9	2.0	2.9	2.8	0	2.5	0	1.4	0
	Rubine	0	0.5	0	0	0	0	0.4	0.4	0.5	0.5	0	0	0	0
	Huizer	0	0	0	0	–	–	0	0	4.3	0	0	0	0	0
Brassica oleracea var. *sabauda*	Hammer	–	–	–	–	0.6	25.2	1.0	41.0	–	–	0	0	0	0
	Groene Putjes	2.0	0	0.7	0	14.3	0	0	0	0	0	0	0	0	0
Brassica oleracea var. *capitata*	Kolos	0	0	0.3	0	0	0	0	0	0.3	0	1.3	0	0	0
	Roem van Enkh.	2.9	0	0	0	–	–	0	0	0	0	0	0	0	0
	Lang. Vr. Herfst	–	–	0	0	–	–	0	0	0	0	0	0	0	0
Brassica oleracea var. *laciniata*	Roem van Hees	0.8	0	0.5	0.2	0	0.8	0	0.8	0	0	0	0	0	0
	Dw. Bl. Scotch	0	0	0	0	0	0	0.9	0	0	0	0	0	0	0
	Westlandse Herfst	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brassica oleracea var. *gongylodes*	Goliath	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0
	Primavera	0	0	0	0	–	–	0	0	0	0	0	0	0	0
Successful combinations per male parent (%)	varieties or accessions species or botanic varieties	28	20	35	10	29	30	31	31	43	10	15	0	8	0
		31	15			30	31			22	3				

Table 4. Analyses of variance on values 1 and 0, assigned respectively to success and failure of seed set in parental combinations. m = matromorphic seeds; h = hybrid seeds.

Source	Dimension	Mean square		F-value	
		m	h	m	h
Between female parents (varieties)	12	0.464	0.227	4.29^{++}	2.99^{++}
Between male parents (varieties, accessions of species)	14	0.225	0.124	2.08^{+}	1.64
Error	151	0.108	0.076		

Specific differences in p.p.a.(b) and p.p.i.a.(b)

Table 3 shows that great differences occur in the frequencies of matromorphic seeds formed when one female parent (a variety) is prickle pollinated by different male parents (varieties, or accessions of species), or when one male parent (variety, or accession of a species) functions as a prickle pollinator for a number of female parents (varieties of botanic varieties). However some parents with relatively great values for general p.p.a. or p.p.i.a. also markedly influence the frequencies of matromorphic seeds formed in such combinations.

Some parental combinations with great values for p.p.a.(b) and p.p.i.a.(b), are Roem van Enkhuizen × Siletta, Huizer × Jobe and Groene Putjes × accession 69022 of *E. sativa*. Between different parental combinations there are also large differences in frequencies of hybrid seeds formed.

Raphanus sativus var. *radicula*				*Brassica nipposi-nica*		*Brassica japonica*		*Camelina sativa*		*Brassica rapa* var. *rapa*		*Crambe abyssinica*				Successful combinations per female parent (%)			
Huiz. Trip.		R. Held.		69012		69004		69050		Novotas		69042		69043		varieties		botanic varieties	
m	h	m	h	m	h	m	h	m	h	m	h	m	h	m	h	m	h	m	h
0.3	0	0.2	0	0	0	0	0	0	0	0	0	0.2	0	0	0	60	20		
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	20	30	16
0	0	0	0	0	0	1.0	0.3	0	0	0	0	0	0	0	0	15	9		
0	0	–	–	0	0	0	0	1.1	1.1	0	0	–	–	–	–	36	32	28	20
0	0	0	1.2	0	0	0	0	0	0	0	0	0	0	0	0	20	7		
1.2	0	0	0	1.3	0	0	0	0	0	0	0	0	0	–	–	35	0		
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	1	15	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2		
0	0.3	0	0	0	0.2	0	0	0	0	0	0	–	–	0	0	13	33		
0	0	0	0	0	0	0	0	0	0	0	0.6	0	0	0	0	7	7	7	13
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0	–	–	0	0	0	0	0	0	0	0	0	0	0	0	7	0	3	0
–	–	0	0	0	0	0	0	0	0	0	0	–	–	–	–	0	1		
15	8	10	10	8	8	8	8	8	8	0	8	9	1	2	1	Mean % of successful combinations		17	10
13	9			8	8	8	8	8	8	0	8	6	1						

Differences in i.p.a.(n)

In Table 5 the i.p.a.(n) is shown for twelve clones of the variety Kolos after prickle pollination by one plant of the variety Huizer's Triplo. Table 5 shows large (significant) differences between clones in seed set after prickle pollination which has not been corrected for germination capacity or numbers of matromorphic seeds. Clone 9 forms a large number of seeds after prickle pollination; seed set in clones 4, 7, 8 and 11 is also considerable.

Differences between the clones in the frequency of matromorphic seeds formed (i.p.a.(n)) are smaller than differences in total seed set, yet they are significant. Matromorphic seeds were not formed on clones 1, 3, 6, 10 and 12; however the frequency of matromorphic seeds in clones 4, 7 and 11, is by comparison, rather large. These differences in i.p.a.(n) between clones from one population are even greater than for instance those for p.p.a. caused by varietal influences (see Table 3).

Because the differences in frequencies of matromorphic seeds are not great in an absolute sense and because of the unequal frequencies of seeds formed by the different plants per clone, there is no point in computing a h^2 value for i.p.a.(n) using variances between and within clones for frequencies of matromorphic seeds formed.

Differences in i.p.i.a.(n)

Table 6 shows the results for i.p.i.a.(n) after prickle pollination of clones by genetically different plants from populations. From Table 6 it appears that there are large (signifi-

Table 5. Seed set of clones of *Brassica oleracea* var. *capitata* cv. Kolos after prickle pollination by one plant of *Raphanus sativus* var. *radicula* cv. *Huizer's Triplo*.

Clone No	Number of prickle pollinated buds	All seeds		Matromorphic seeds	
		number	number per 100 pr. poll. buds	number	number per 100 pr. poll. buds.
1	695	0	0.00	0	0.00
2	1250	2	0.16	1	0.08
3	1312	1	0.08	0	0.00
4	1282	7	0.55	5	0.39
5	952	2	0.21	1	0.11
6	683	0	0.00	0	0.00
7	756	5	0.66	5	0.66
8	991	5	0.50	2	0.20
9	813	20	2.46	1	0.12
10	612	0	0.00	0	0.00
11	756	4	0.53	4	0.53
12	464	1	0.22	0	0.00

cant) differences in i.p.i.a.(n) among *E. sativa* plants from one population, with respect to one clone of the variety Kolos. These differences among *E. sativa* plants from the same population with respect to one clone of the variety Hybride 69002 are smaller and not significant. In both parental combinations some male parent plants were present which did not induce parthenogenesis, but also plants which gave rise to a relatively high frequency of matromorphic seeds. Plant 11 of the *E. sativa* population does not induce the development of matromorphic seeds, however, hybrids (genomes: X (*B.ol.*) + X (*E.sat.*)) were formed in a high frequency. In many parental combinations mentioned before (see Table 3), matromorphics and hybrids were formed on the same plant, here congruity (HOGENBOOM, 1973) and induction of parthenogenesis are not associated.

In the combination Kolos × Huizer's Triplo the differences in i.p.i.a.(n) between the six male parent plants tested are rather slight and not significant.

The h^2 value for i.p.i.a.(n) was not calculated because of low seed set and uneven frequencies of matromorphic seeds formed on the different plants of a clone.

In testing differences in seed set for significance, the Test for significance of the difference between two Poisson variables was used (PEARSON & HARTLEY, 1954).

So it appears from Table 3, 5 and 6 that considerable differences in p.a. and p.i.a. may occur between genera, species, botanic varieties, varieties or accessions, and plants from a variety or accession.

The genetics of the p.a. and the p.i.a. is probably rather complicated, because a range of processes in both the female and the male parent will eventually result in the development of matromorphic seeds. In the female parent for instance, unreduced egg-cells may have to be present (MACKAY, 1972) which originate from a deviating meiosis and which should develop parthenogenetically; endosperm probably also develops, perhaps autonomously or after fusion of polar nuclei with generative nuclei.

Table 6. Seed set of clones after prickle pollination by different plants of a population.

Female parent	Male parent		Number of prickle pollinated buds	All seeds		Matromorphic seeds		Hybrid seeds	
				number	number per 100 pr. poll. buds	number	number per 100 pr. poll. buds	number	number per 100 pr. poll. buds
Brassica oleracea	*Eruca sativa*	pl. 1	1215	2	0.16	1	0.08	0	0.00
var. *capitata*	accession 69039	pl. 2	1094	4	0.37	4	0.37	0	0.00
cv. Kolos		pl. 3	1395	1	0.07	1	0.07	0	0.00
		pl. 4	1195	8	0.67	8	0.67	0	0.00
		pl. 5	1076	2	0.19	2	0.19	0	0.00
		pl. 6	1084	0	0.00	0	0.00	0	0.00
Brassica oleracea	*Eruca sativa*	pl. 7	870	3	0.34	2	0.23	1	0.11
var. *gemmifera*	accession 69039	pl. 8	913	3	0.33	2	0.22	1	0.11
cv. Hybride 69002		pl. 9	812	2	0.25	2	0.25	0	0.00
		pl.10	776	0	0.00	0	0.00	0	0.00
		pl.11	805	39	4.84	0	0.00	27	3.35
		pl.12	789	1	0.13	1	0.13	0	0.00
Brassica oleracea	*Raphanus sativus*	pl. 1	1172	1	0.09	0	0.00	0	0.00
var. *capitata*	var. *radicula* cv.	pl. 2	1150	1	0.09	1	0.09	0	0.00
cv. Kolos	Huizer's Triplo	pl. 3	970	0	0.00	0	0.00	0	0.00
		pl. 4	1299	0	0.00	0	0.00	0	0.00
		pl. 5	1000	3	0.30	3	0.30	0	0.00
		pl. 6	1339	3	0.22	3	0.22	0	0.00

The pollen of the prickle pollinators should germinate and pollen tubes should grow through the style into the embryosac (NOGUCHI, 1928; RÖBBELEN, 1965). Results of research on the development of matromorphic embyos will be published later.

Research on the genetics of the parthenogenetic ability was carried out as follows. Crosses were made between parents of which one (P1 = Hybride 69002 and Kolos respectively) can produce matromorphic seeds in a high frequency and the other (P2 = Rubine respectively Langed. Vroege Herfst) produces matromorphic seeds in a very low frequency. The parents and the F1's were prickle pollinated by one plant respectively of the variety Jobe and the accession 69039 of *E. sativa*. As however the frequencies of matromorphic seeds formed in the P_1 (clone), $P_1\otimes$, $P_2\otimes$ and F_1 ($P_1 \times P_2$) populations were small, it was difficult to draw conclusions about the genetics of the p.p.a.(n). However the results of these investigations seem to indicate that a good p.p.a.(n) is inherited non-recessively. This agrees with earlier statements by other research workers (RUTISHAUSER, 1967).

CONCLUSIONS

The i.p.a. and the i.p.i.a. may have a large effect on the frequencies of matromorphic seeds formed after prickle pollination. Also specific and general determined differences in p.p.a. or p.p.i.a. may greatly influence the frequency of matromorphic seeds formed. Therefore, if from a population of *B. oleracea* L. matromorphic plants have to be obtained in a high frequency it is important to detect, by using a population of prickle pollinators, parental combinations with a good p.p.a., p.p.i.a., i.p.a. and i.p.i.a.

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MATROMORPHY IN *BRASSICA OLERACEA* L. III. THE INFLUENCE OF TEMPERATURE, DELAYED PRICKLE POLLINATION AND GROWTH REGULATORS ON THE NUMBER OF MATROMORPHIC SEEDS FORMED

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SUMMARY

Research was carried out into influence of extraneous factors on the frequency of matromorphic seeds. Because of a low seed set in different parental combinations, few significant differences were found. It appeared, however, that temperature could positively influence the frequency of matromorphic and hybrid seeds. Delayed prickle pollination and application of GA3 also increased the number of seeds.

INTRODUCTION

There is a large variation in frequency of matromorphic seeds formed in different parental combinations. This variation may originate from differences in parthenogenetic ability and parthenogenesis inducing ability (EENINK, 1974). In addition environmental factors may be involved (NISHI et al., 1964; RÖBBELEN, 1965).

The ability of an egg-cell to develop into a matromorphic embryo may be influenced by temperature. Whether this will actually occur may also depend on temperature. With *Hieracium*, for instance, temperature can disturb meiosis (GENTCHEFF & GUSTAFFSON, 1940; GUSTAFFSON & NYGREN, 1946). As a result of the absence of meiosis, diploid cells are formed in varying frequencies which may cause changes in the incidence of apomixis. If matromorphic embryos develop from egg-cells which are diploid as a result of a deviation in pre-meiotic or meiotic divisions, then the temperature before and/or during meiosis and the temperature after meiosis may influence the number of matromorphic seeds formed. If they develop from egg-cells which are diploid as a result of deviations during or after gametogenesis (e.g. during or after prickle pollination) then only the temperature in this period may influence the number of matromorphic seeds formed.

Delayed (prickle) pollination may increase the frequency of parthenogenesis. In *Triticum* more parthenogenetically developed seeds were formed after a delayed pollination (KIHARA, 1940; KIHARA et al., 1942; TSUNEWAKI et al., 1968); already before delayed pollination is carried out, the embryo starts developing parthenogenetically. After pollination the endosperm develops.

The application of growth regulators may also influence positively the number of matromorphic seeds formed. According to NISHI et al. (1964) the frequency in which

matromorphic seeds were formed, increased after application of NAA to prickle pollinated plants.

This paper reviews the results of a research into the influence of the above mentioned extraneous factors on the number of matromorphic seeds formed.

METHODS AND MATERIALS

The investigations were carried out in a phytotron at constant temperatures of 10, 14 and 17 °C, a daylength of 16 hours and a relative humidity of about 80%.

Temperature. Research into the temperature effect on the number of matromorphic seeds formed was carried out with plants of a clone functioning as a female parent and one plant as a prickle pollinator. The parental combinations used were *Brassica oleracea* L. var. *capitata* L. cv. Kolos × *Raphanus sativus* L. var. *radicula* PERS. cv. Huizer's Triplo, *B. oleracea* L. var. *gemmifera* (DC.) SCHULZ cv. Hybride 69002 × *Eruca sativa* MILL. accession 69039 and *B. oleracea* L. var. *sabauda* (L.) SCHULZ cv. Hammer × *E. sativa* MILL. accession 69039. The number of plants per female parent clone varied between 10 and 15 per temperature.

The influence of temperature before and/or during meiosis on the number of matromorphic seeds formed was investigated as follows. Equal numbers of female parent plants were placed at 10, 14 and 17 °C. When the plants started flowering they were transferred to 14 °C. All buds with egg-cells resulting from pre-meiosis and meiosis at the alternative temperature, were prickle pollinated, the plants stayed at 14 °C till the harvest of the siliques.

Research on the influence of temperature after meiosis, during or after prickle pollination on the number of matromorphic seeds formed was carried out as follows. Pre-meiosis and meiosis of female parent plants occurred at 14 °C. When the plants started flowering, equal numbers were distributed over the temperatures of 10, 14 and 17 °C. At these temperatures buds with egg-cells resulting from pre-meiosis and meiosis at 14 °C were prickle pollinated; the plants stayed at these temperatures till the harvest of the siliques.

Delayed prickle pollination. With the parental combinations *B. oleracea* var. *capitata* cv. Kolos × *R. sativus* var. *radicula* cv. Huizer's Triplo, *B. oleracea* var. *gemmifera* cv. Hybride 69002 × *E. sativa* accession 69039 and *B. oleracea* var. *sabauda* cv. Hammer × *E. sativa* accession 69039 ten clonal plants of each female parent at 14 °C were prickle pollinated by one male parent plant. Prickle pollinations were made between 0 and 144 hours after emasculation. The prickle pollinated buds were equal in size at the moment of emasculation, about one day before flowering. The times of (delayed) prickle pollination were evenly distributed over plants and time.

In order to investigate the causes of a possible influence of delayed prickle pollination on the number of matromorphic seeds, pollen tube growth in prickle pollinated pistils was studied. Differences in numbers or lengths of pollen tubes between delayed and non-delayed prickle pollination might be of importance. Flower buds of plants of one clone of the variety Hybride 69002 were prickle pollinated 0 to 5 days after emasculation by one plant of *Brassica rapa* L. var. *rapa* cv. Jobe. The pistils were harvested

one day after prickle pollination, macerated for one hour in NaOH (1N) at 60°C, stained in Wasserblau for one hour (for the composition of the Wasserblau solution, see VISSER, 1973) and investigated under a UV-microscope. The reason why no pollen tube growth of the two earlier mentioned prickle pollinators (Huizer's Triplo and *E. sativa* accession 69039) was studied is that no tubes from pollen of these pollinators could be observed in pistils of prickle pollinated buds. These pollen tubes must have been present in view of the fact that many hybrid seeds developed.

Growth regulators. Plants of one clone of *B. oleracea* var. *gemmifera* cv. Hybride 69002 at 14°C functioning as female parents were prickle pollinated by one plant of *E. sativa* accession 69039. The growth regulators were dissolved in distilled water (NAA, 0 and 1 ppm; GA3, 0, 1 and 10 ppm; Ethrel, 0 and 100 ppm.) and applied at the time of prickle pollination. Per prickle pollinated bud, one drop of a solution was applied on the ovary. Five female parent plants were used per growth regulator per concentration. The reason why no more different concentrations of NAA and Ethrel were used is that insufficient clonal plant material was available.

In testing differences in seed set for significance, the Test for significance of the difference between two Poisson variables was used (PEARSON & HARTLEY, 1954).

For material and methods not mentioned here reference is made to EENINK (1974).

RESULTS AND DISCUSSION

The influence of temperature

The temperature before and during meiosis. Table 1 shows the frequencies of matromorphic and hybrid seeds formed in the three groups of plants (placed at 10, 14 and 17°C during pre-meiosis and meiosis) of the different parental combinations. The frequencies of seeds formed (matromorphics and hybrids) are low in all parental combinations tested. In Kolos matromorphic seeds were formed in a low frequency in plants which were placed at 14 and 17°C during pre-meiosis and meiosis. The plants which were placed at 10°C did not produce matromorphic seeds at all. Of the variety Hybride 69002 the plants at 10°C during pre-meiosis and meiosis formed significantly more matromorphic seeds per 100 prickle pollinated buds than those placed at 14 or 17°C. Hybrid seeds were formed in low frequencies by the three groups of plants of this variety. In the variety Hammer no matromorphic seeds were formed.

The temperature after meiosis, during or after prickle pollination. Table 2 shows the frequencies of matromorphic and hybrid seeds formed in three groups of plants of different parental combinations at 10, 14 and 17°C, respectively. The plants of the variety Kolos formed significantly more matromorphic seeds per 100 prickle pollinated buds at 14°C than at 10 and 17°C. Hybride 69002 formed matromorphic seeds in a very low frequency and no significant differences occur between frequencies of matromorphic seeds formed at different temperatures. At 17°C, however, significantly more hybrid seeds were formed per 100 prickle pollinated buds than at 14 or 10°C. The variety Hammer formed no matromorphic seeds at all. The frequencies of hybrid seeds formed, however, are considerable. At 14 and 17°C (significantly) more hybrid seeds were formed than at 10°C.

Table 1. Seed set of clones of *Brassica oleracea* at 14 °C, placed at different temperatures during pre-meiosis and meiosis.

Parental combination	Number of prickle poll. buds	Matromorphic seeds		Hybrid seeds	
		number	number per 100 pr. poll. buds	number	number per 100 pr. poll. buds
Temperature before and during meiosis 10 °C					
A	3940	0	0	0	0
B	1388	10	0.72	1	0.07
C	2370	0	0	0	0
Temperature before and during meiosis 14 °C					
A	3147	5	0.16	0	0
B	1461	0	0	3	0.21
C	1395	0	0	0	0
Temperature before and during meiosis 17 °C					
A	2472	3	0.12	0	0
B	1379	1	0.07	7	0.51
C	1761	0	0	1	0.06

A = *Brassica oleracea* var. *capitata* cv. Kolos × *Raphanus sativus* var. *radicula* cv. Huizer's Triplo.
B = *Brassica oleracea* var. *gemmifera* cv. Hybride 69002 × *Eruca sativa* accession 69039.
C = *Brassica oleracea* var. *sabauda* cv. Hammer × *Eruca sativa* accession 69039.

Table 2. Seed set of clones of *Brassica oleracea* at different temperatures, placed at 14 °C before and during meiosis.

Parental combination	Number of prickle poll. buds	Matromorphic seeds		Hybrid seeds	
		number	number per 100 pr. poll. buds	number	number per 100 pr. poll. buds
Temperature after meiosis, during and after prickle pollination 10 °C					
A	3933	0	0	0	0
B	2058	2	0.10	1	0.05
C	1855	0	0	19	1.02
Temperature after meiosis, during and after prickle pollination 14 °C					
A	3888	13	0.33	0	0
B	1251	0	0	6	0.48
C	1900	0	0	48	2.53
Temperature after meiosis, during and after prickle pollination 17 °C					
A	3776	2	0.05	0	0
B	1924	0	0	23	1.20
C	1729	0	0	30	1.74

A = *Brassica oleracea* var. *capitata* cv. Kolos × *Raphanus sativus* var. *radicula* cv. Huizer's Triplo.
B = *Brassica oleracea* var. *gemmifera* cv. Hybride 69002 × *Eruca sativa* accession 69039.
C = *Brassica oleracea* var. *sabauda* cv. Hammer × *Eruca sativa* accession 69039.

From the above results it appears that temperature, before and during as well as after meiosis had some influence on the frequency of matromorphic seeds formed. Because of a low seed set, however, few significant differences were present. Influence of the temperature before or during meiosis suggests a connection between processes during pre-meiosis and/or meiosis and the frequency of parthenogenesis; matromorphic embryos may have a diploid number of chromosomes caused by irregularities in pre-meiosis or meiosis and will then be heterozygous. The influence of temperature after meiosis, during and/or after prickle pollination may point to a doubling of the haploid number of chromosomes in an egg-cell which eventually develops into a matromorphic embryo. In such a case the matromorphic embryo would be homozygous at all loci.

Research carried out with the three above mentioned parental combinations into the influence on seed set of high temperatures (23 and 26 °C) and temperature shocks (4 °C) before, during and after meiosis did not yield reliable figures, owing to a very low seed set. Research into influences of different day and night temperatures (17 °C day, 10 °C night; 14 °C day, 10 °C night) also failed from the same cause.

The influence of delayed prickle pollination

Table 3 shows the seed set after delayed prickle pollination. The different times of delay are summarized in categories of 24 hours. From Table 3 it appears that delayed

Table 3. Seed set of clones of *Brassica oleracea* after delayed and non-delayed prickle pollination.

Prickle pollination in hours after emasculation	Number of prickle poll. buds	Matromorphic seeds		Hybrid seeds	
		number	number per 100 pr. poll. buds	number	number per 100 pr. poll. buds
Brassica oleracea var. *capitata* cv. Kolos × *Raphanus sativus* var. *radicula* cv. Huizer's Triplo					
0	244	0	0	0	0
1–24	96	0	0	0	0
25–48	922	2	0.22	0	0
49–72	751	2	0.27	0	0
73–96	193	1	0.52	0	0
>96	91	0	0	0	0
Brassica oleracea var. *gemmifera* cv. Hybride 69002 × *Eruca sativa* accession 69039					
0	146	0	0	1	0.68
1–24	212	0	0	3	1.42
25–48	463	0	0	36	7.78
49–72	424	1	0.24	18	4.25
73–96	25	0	0	12	48.00
>96	139	0	0	9	6.47
Brassica oleracea var. *sabauda* cv. Hammer × *Eruca sativa* accession 69039					
0	104	0	0	0	0
1–24	81	0	0	0	0
25–48	449	0	0	29	6.46
49–72	514	1	0.19	48	9.34
73–96	368	12	3.26	36	9.78
>96	–	–	–	–	–

prickle pollination had a positive effect on the frequency of seeds formed. None of the varieties formed matromorphic seeds when prickle pollinated less than 48 hours after emasculation. When prickle pollination was made between 48 hours and 96 hours after emasculation, some matromorphic seeds developed in Kolos. Hybride 69002 formed only one matromorphic seed after delayed prickle pollination. In Hammer prickle pollination more than 48 hours after emasculation resulted in a rather high frequency of matromorphic seeds.

The differences between delayed and non-delayed prickle pollination in the frequencies of hybrid seeds formed in the different parental combinations are very large. Pollination delayed by 48 hours or more resulted in high frequencies of hybrid seeds both in Hybride 69002 and Hammer. Prickle pollinations of Hammer delayed by more than 96 hours were not made because of a shortage of clonal plants.

It is not likely that a higher frequency of matromorphic seeds is caused by premature embryo development, because after delayed prickle pollination the frequency of both hybrid and matromorphic seeds increased. Besides, a research using microtome preparations of egg-cells, embryos and endosperm in siliques after delayed prickle pollination also showed that autonomous premature embryo development did not occur.

Table 4 shows the growth of tubes of pollen from a plant of Jobe, after delayed and non-delayed prickle pollination, in pistils of plants of a clone of Hybride 69002. The length of the pollen tubes is shown on the scale of a measuring ocular (one unit = 166 μm). Table 4 shows that between delayed and non-delayed prickle pollination remarkable differences occurred in number of pollen tubes per pistil; means of pollen tube lengths per pistil were randomly distributed over the groups representing different moments of prickle pollination. Prickle pollination two or more days after emasculation resulted in larger numbers of pollen tubes than prickle pollination immediately after emasculation. The best moment was three or four days after emasculation. This agrees with results for seed set after delayed and non-delayed pollination (see Table 3 and 4). Therefore the larger numbers of pollen tubes in pistils after delayed prickle pollination may be responsible for the increase in seed set.

Table 4. Number and length of tubes of pollen from one plant of *Brassica rapa* var. *rapa* cv. Jobe in pistils of plants of a clone of *Brassica oleracea* var. *gemmifera* cv. Hybride 69002. Means are based on observations of pollen tubes in 20 pistils.

Prickle pollination in days after emasculation	Mean number of pollen tubes per pistil	Mean length of pollen tubes per pistil
0	4	10
1	2	18
2	6	8
3	17	19
4	17	16
5	8	13

The influence of growth regulators

Table 5 shows the results for seed set after application of growth regulators. The figures for seed set after application of Ethrel are not given in this table, because nearly all plants receiving this growth regulator died prematurely. From Table 5 it appears that application of both 1 and 10 ppm GA3 solutions had a positive effect on the frequency of matromorphic seeds. After application of 1 ppm NAA solution no matromorphic seeds were formed, nor were such seeds formed by any of the control plants. No hybrid seeds were obtained.

The reason for the increased seed set after application of GA3 is not known. Perhaps larger numbers of siliques, formed by plants treated with GA3, influence positively the frequency of matromorphic seeds. These differences between treated and untreated plants, however, are rather slight, probably because of the natural parthenocarpic ability of the *Brassica* plants. Therefore a direct influence of GA3 on the development of the ovules or an influence on pollen tube growth may be more likely.

Internal factors

Besides external factors, internal factors different from parthenogenetic ability and parthenogenesis inducing ability also influence the frequencies of matromorphic and hybrid seeds formed. Within plants of *Brassica oleracea* investigated, no significant differences could be observed between different regions (upper, lower part; different side branches etc.). Within siliques, however, significant differences occurred between the upper and lower halves of the siliques in numbers of seeds formed, as shown in Table 6.

The cause of the differences shown in Table 6 is not known. This uneven distribution disappeared with (hybrid) seeds obtained from delayed pollination. It is not

Table 5. Matromorphic seeds formed by prickle pollinated plants of one clone of *Brassica oleracea* var. *gemmifera* cv. Hybride 69002 after application of growth regulators.

Growth regulator	Concentration in ppm	Number of pr. poll. flower buds	Matromorphic seeds	
			number	number per 100 pr. poll. flower buds
GA3	1	468	2	0.43
GA3	10	720	6	0.83
NAA	1	406	0	0
None (Control)	0	1033	0	0

Table 6. Numbers of matromorphic and hybrid seeds formed in the upper and the lower half of the siliques from plants of botanic varieties of *Brassica oleracea* after (non-delayed) prickle pollination with different male parents.

Matromorphic seeds				Hybrid seeds			
upper half	lower half	X^2 (1:1)	P	upper half	lower half	X^2 (1:1)	P
81	31	22.32	<0.001	203	86	47.36	<0.001

likely that differences in the time when the pollen tubes reach the ovules play a role, because the upper and lower ovules were reached at about the same time. Perhaps differences in maturity stage (SCHWEMMLE, 1957) of upper and lower ovules cause the distribution of seeds shown in Table 6; variations in concentrations of (a) growth regulator(s) might be responsible.

CONCLUSIONS

The number of matromorphic seeds formed can be influenced by manipulating extraneous factors. Temperature before, during as well as after meiosis seems to influence the seed set whereas after delayed prickle pollination the frequency of seeds was higher than after non-delayed prickle pollination. Application of GA3 solutions also positively influenced the frequency of matromorphic seeds formed. Therefore, if from a population, matromorphic plants have to be obtained in a high frequency it is advantageous to make use of such extraneous factors.

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MATROMORPHY IN *BRASSICA OLERACEA* L. IV. FORMATION OF HOMOZYGOUS AND HETEROZYGOUS DIPLOID PRODUCTS OF GAMETOGENESIS AND QUALITATIVE GENETICAL RESEARCH ON MATROMORPHIC PLANTS

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SUMMARY

An account is given of possible deviations in cell or nuclear divisions before, during or after meiosis resulting in homozygous or heterozygous diploid products of gametogenesis.

After research on matromorphic plants for qualitative characters it appeared that part of the plants were heterozygous for these characters.

From the results of this research it may be concluded that it is not unlikely that matromorphic embryos develop from egg-cells which were diploid as a result of different deviations in cell or nuclear divisions.

INTRODUCTION

The level of homozygosity of matromorphic plants is determined by the moment (before, during or after meiosis) at which deviations in cell or nuclear divisions occur resulting in diploid products of gametogenesis which may develop into matromorphic embryos.

By qualitative genetical research the homozygosity of matromorphic plants can be investigated. NISHI et al. (1964) and RÖBBELEN (1965), who investigated matromorphic plants of *Raphanus* and *Brassica* respectively, found that all these plants were homozygous for a qualitative character (skin colour and leaf colour respectively). They concluded that the matromorphic plants were completely homozygous and were diploid as a result of doubling of chromosomes after sporogenesis. TOKUMASU (1965), however, demonstrated that matromorphic *Brassica* plants occurred which were heterozygous for the character 'split style'. From the distribution of the matromorphic plants over the three genotypes for this character, he concluded that the matromorphic embryos developed from egg-cells which were diploid as a result of pre-meiotic endomitosis followed by a normal meiosis with random chromosome pairing. HODGKIN & REDFERN (1971) found matromorphic *Brassica* plants with heterozygous genotypes for glossy leaves and incompatibility. MACKAY (1972) also found matromorphic *Brassica* plants with heterozygous genotypes for incompatibility; he suggested that the matromorphic embryos developed from egg-cells which were diploid as a result of a deviating meiosis.

This paper reviews possible deviations in cell or nuclear divisons before, during or after meiosis, which may result in the development of homozygous or heterozygous

From the resulting matromorphic plants the genotypes for the above characters were identified.

The genotypes for incompatibility of the matromorphic plants were identified by making test crosses between matromorphic plants and plants with homozygous genotypes of the S-alleles present in the prickle pollinated female parent. In order to identify these genotypes, first the behaviour of the S-alleles in homozygous and heterozygous genotypes, in pollen and stigma was analysed by using diallel crosses between control plants of these genotypes. The (in)compatibility reaction was investigated by using UV-microscopy (for a description of this method, see EENINK, 1974).

From analyses of F_2's from crosses between plants with glossy leaves and plants with non-glossy leaves, it appeared that glossy/non-glossy in this cross was determined by one gene pair (phenotype-genotypes: non-glossy, *GG* or *Gg* and glossy, *gg*) or by two or more closely linked gene pairs.

Analyses of the number of gene pairs for anthocyanin and leaf curl by using F_2's from crosses between anthocyanin-containing plants without curled leaves and anthocyanin-free plants with curled leaves showed that both characters were governed by more than one dominant gene (anthocyanin A_1–A_x, curled leaf C_1–C_x).

RESULTS AND DISCUSSION

Table 2 shows the seed set per parental combination and the distribution of plants over the different genotypes for incompatibility, glossy leaves and anthocyanin and leaf curling.

It appears that considerable differences occur in the frequency of seeds formed per parental combination. Some combinations do not form seeds at all, others only form matromorphic or hybrid seeds.

Both homozygous and heterozygous genotypes for incompatibility or glossy leaves are present in matromorphic plants. The genotypes for anthocyanin and leaf curl were heterozygous; a progeny of the matromorphic plant obtained from selfing included plants with and without anthocyanin and curled leaves.

From the presence of both homozygous and heterozygous genotypes for the investigated characters it appears that matromorphic embryos do not (only) develop from egg-cells which were diploid as a result of doubling of chromosomes during or after gametogenesis. These results do not agree with those obtained by NISHI et al. (1964) and RÖBBELEN (1965). Nor do matromorphic embryos (only) develop from egg-cells which were diploid as a result of pre-meiotic endomitosis and autobivalent formation at meiosis (quadrivalent formation or random pairing is less likely). The hypothesis of TOKUMASU (1965), therefore, appears to be inapplicable for the botanic varieties of *B. oleracea* investigated here.

It is not unlikely that matromorphic embryos develop from egg-cells which are diploid as a result of different deviations in cell or nuclear divisions. Plausible hypotheses for the formation of diploid egg-cells are the absence of meiosis and the failure of the first or second meiotic division. The failure of the second meiotic division could result in homozygous genotypes for loci closely situated at the centromere, owing to which no crossing-over between this locus and the centromere occurs. This might be the explanation for the occurrence of only homozygous genotypes of matromorphic

Table 2. Genotypes of qualitative characters in matromorphic plants obtained from different prickle pollinated female parent plants. – = figures not known (relate to > 5000 prickle poll. buds).

Female parent	Character	Genotype	Number of prickle poll. flower buds	Matromorphic seeds		Hybrid seeds		Distribution of matromorphic plants over genotypes
				number	number per 100 prickle poll. flower buds	number	number per 100 prickle poll. flower buds	
Brassica oleracea var. *capitata* cv. Kolos	incompatibility	SaSb	–	11	–	–	–	5 SaSa 6 SaSb 0 SbSb
Brassica oleracea var. *gemmifera* cv. Hybride 69002	incompatibility	ScSd	–	29	–	–	–	4 ScSc 11 ScSd 14 SdSd
Brassica oleracea var. *gemmifera* F1 (SeSf)	incompatibility	SeSf	5639	1	0.02	0	0	0 SeSe 1 SeSf 0 SfSf
Brassica oleracea var. *gemmifera* F1 (SeSg)	incompatibility	SeSg	1937	0	0	8	0.41	
Brassica oleracea var. *sabauda* cv. Hammer	incompatibility	ShSi	–	11	–	–	–	1 ShSh 8 ShSi 2 SiSi
Brassica oleracea var. *gemmifera* F1 (Hybride 69002 × plant 101.2)	glossy leaves	Gg	1267	0	0	0	0	
Brassica oleracea var. *gemmifera* F1 (Hydride 69002 × plant 115.7)	glossy leaves	Gg	1016	0	0	0	0	
Brassica oleracea var. *gemmifera* F1 (Rubine × plant 101.2)	glossy leaves	Gg	4706	0	0	31	0.66	
Brassica oleracea var. *gemmifera* F1 (Plant 115.7 × Stiekema no. 1)	glossy leaves	Gg	3146	2	0.06	17	0.54	0 GG 1 Gg 1 gg
Brassica oleracea var. *gemmifera* F1 (Plant 115.7 × Huizer laat)	glossy leaves	Gg	913	1	0.11	0	0.00	0 GG 1 Gg 0 gg
Brassica oleracea var. *gemmifera* × *B. oleracea laciniata* F1 (Rubine × Roem van Hees)	anthocyanin curled leaf	A1a1–Axax, C1c1–Cxcx	1939	1	0.05	102	5.26	1 heterozygous for anthocyanin and curled leaf

plants for qualitative characters investigated by NISHI et al. (1964) and RÖBBELEN (1965). Diploid embryos may also develop as a result of pre-meiotic endomitosis or a doubling of chromosomes during or after gametogenesis.

Because of these different potentialities with respect to the formation of diploid products of gametogenesis it is impossible to determine the time of the chromosome doubling or non-reduction on the basis of distributions of matromorphic plants over different genotypes for qualitative characters.

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MATROMORPHY IN *BRASSICA OLERACEA* L. V. STUDIES ON QUANTITATIVE CHARACTERS OF MATROMORPHIC PLANTS AND THEIR PROGENY

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SUMMARY

A theoretical review is given of genotypical means and variances of matromorphic populations which developed in different ways.

From combining ability analyses of variance and comparisons of means and variances of quantitative characters of matromorphic and control plants or their progeny it is concluded that matromorphic plants do develop in different ways.

It seems likely that matromorphic embryos develop from egg-cells which were diploid as a result of pre-meiotic endomitosis (followed by autobivalent formation) or absense of meiosis and from egg-cells diploid as a result of failing of the first or second meiotic division. Homozygous matromorphic plants appear not to occur.

INTRODUCTION

The homozygosity of matromorphic plants can be investigated by qualitative genetical research (NISHI et al., 1964; RÖBBELEN, 1965; TOKUMASU, 1965; HODGKIN & REDFERN, 1971; MACKAY, 1972; EENINK, 1974). However, because of a lack of knowledge of the location on the chromosomes of the genes concerned and depending on the way of origination of diploid products of gametogenesis (EENINK, 1974) incorrect conclusions can be drawn as to the homozygosity of the matromorphic plants.

A better impression of homozygosity of matromorphic plants may be obtained by comparisons of means and/or variances of quantitative characters. Perhaps an impression of the way of origination of the matromorphic plants can also be obtained. This is illustrated by the following theoretical review in which matromorphic populations, formed in different ways (EENINK, 1974), are compared with control populations. The latter were populations of the female parent varieties and populations obtained by selfing of the female parents. Table 1 shows genotypical means and variances of such matromorphic and control populations. The (female) parents from which they originated were respectively a hybrid variety and an open pollinated variety. The open pollinated variety was assumed to be in a Hardy-Weinberg equilibrium with $p = q = 0.5$. The calculations have been made according to an additive-dominance model (MATHER & JINKS, 1971) for one locus. The genetic parameters d and h indicate respectively half the difference of the genotypic values of both homozygotes and the difference between the genotypic value of the heterozygote and the midparent.

From this table it appears that genotypical means and variances of matromorphic populations are greater than, equal to, or smaller than those of the control popula-

Table 1. Genotypical means and variances of different (matromorphic) populations. In this model: AA = +d, Aa = +h, aa = -d.

Population of matromorphic plants diploid as a result of:	Female parent is a hybrid variety						
	genotype frequency			genotypical population mean	variance (σ^2)	$\sigma^2 \geqslant \sigma^2$ variety	$\sigma^2 \geqslant \sigma^2$ variety ⊗
	AA	Aa	aa				
pre-meiotic endomitosis (autobivalents), or no meiosis, or failing first meiotic division (without pairing), or failing second meiotic division (1 × crossing-over) (female parent population)	0	1	0	h	0	always ($\sigma^2 = \sigma^2$variety)	never
failing first meiotic division (1 × crossing-over), or failing second meiotic division (2 × crossing-over) (female parent ⊗ population)	1	2	1	$^1/_2$h	$^1/_2$d^2 + $^1/_4$h^2	always	always ($\sigma^2 = \sigma^2$variety ⊗)
pre-meiotic endomitosis (quadrivalents, or random pairing, no crossing-over)	1	4	1	$^2/_3$h	$^1/_3$d^2 + $^2/_9$h^2	always	never
failing first meiotic division (2 × crossing-over)	1	6	1	$^3/_4$h	$^1/_4$d^2 + $^3/_{16}$h^2	always	never
failing second meiotic division (no crossing-over), or normal meiosis and chromosome doubling during or after gametogenesis	1	0	1	0	d^2	always	h $\leqslant$ d$\sqrt{2}$

tions. This depends on the type of the female parent population (hybrid or open pollinated variety), the formation of matromorphic embryos and the ratio of h to d. If in the population of the open pollinated variety $p \neq q \neq 0.5$ (this is likely for many loci), great changes in means and variances of matromorphic and control populations may occur. The relative genotype frequency which can be expected to occur in diploid products of gametogenesis will probably be a combination of the relative genotype frequencies mentioned in the table (for instance for a certain locus 0, 1 and 2 times of crossing-over between this locus and the centromere may occur).

KAKIZAKI (1924) compared matromorphic plants with plants of the female parent variety and found that matromorphic plants showed no 'inbreeding depression'.

An impression of the level of heterozygosity and maybe of the way of origination of the matromorphic plants can also be obtained by comparisons of means and variances of quantitative characters of progenies from matromorphic plants and control plants (I1's). For if matromorphic plants are more (less) heterozygous for a quantitative character than the control plants, their progenies obtained by selfing or diallel crosses will show a greater (smaller) variation within the progeny than such progenies from control plants. The means for quantitative characters of progenies obtained by

Female parent is an open pollinated variety

genotype frequency			genotypical population mean	variance (σ^2)	$\sigma^2 \geqslant \sigma^2$ variety	$\sigma^2 \geqslant \sigma^2$ variety $\otimes$
AA	Aa	aa				
1	2	1	$^1/_2h$	$^1/_2d^2+^1/_4h^2$	always ($\sigma^2=\sigma^2$ variety)	$h \geqslant 2d$
3	2	3	$^1/_4h$	$^3/_4d^2+^3/_{16}h^2$	$h \leqslant 2d$	always ($\sigma^2=\sigma^2$ variety $\otimes$)
1	1	1	$^1/_3h$	$^2/_3d^2+^2/_9h^2$	$h \leqslant d\sqrt{6}$	$h \geqslant {}^2/_5d\sqrt{15}$
5	6	5	$^3/_8h$	$^5/_8d^2+^{15}/_{64}h^2$	$h \leqslant 2d\sqrt{2}$	$h \geqslant {}^2/_3d\sqrt{6}$
1	0	1	0	d^2	$h \leqslant d\sqrt{2}$	$h \leqslant {}^2/_3d\sqrt{3}$

selfing or diallel crosses of matromorphic plants and the means for these characters of such progenies from control plants may differ if the level of heterozygosity of matromorphic and control plants differs significantly. The significance of such differences in means, however, also depends on the mean h to d ratio for the loci concerned. This type of research was done by NISHI et al. (1964). They found that a progeny obtained by selfing of a matromorphic plant showed inbreeding depression.

This paper describes the results of a research on quantitative characters of matromorphic plants and control plants and their progeny.

MATERIAL AND METHODS

Comparisons of means and variances of stem length and head weight of matromorphic and control populations. Matromorphic and control populations were obtained from five varieties of *Brassica oleracea* L.: var. *capitata* L. cv. Kolos, var. *capitata* L. cv. Roem van Enkhuizen, var. *gemmifera* (DC.) SCHULZ cv. Hybride 69002, var. *sabauda* (L.) SCHULZ cvs. Hammer and Putjes. The control populations were formed after self-fertilization of plants which also formed matromorphics; sometimes a population of

the female parent variety was also used as a control. Matromorphic and control populations were planted in the field according to a randomized design (single plant randomization). Observations were made at the end of the growing period. Stem length (from stem base to growing point) or head weight were measured. Comparisons between matromorphic and control populations were made for means and variances of these characters.

Comparisons of means and variances of stem lenght of sum-populations obtained from diallel crosses between matromorphic plants and between control plants. After prickle pollination of the experimental hybrids 258, 407, 427 and 432 of *B. oleracea* L. var. *gemmifera* (DC.) SCHULZ, matromorphic plants (m) were obtained. Control plants (c) were obtained after selfing these experimental hybrids. Between 11 matromorphic plants from the same and from different female parents (258 m1, 258 m2, 407 m1, 407 m2, 407 m3, 427 m1, 432 m1, 432 m2, 432 m3, 432 m4, 432 m5) diallel crosses including selfs, without reciprocals, were made. Also between 9 control plants obtained from the same and from different parents (258 c1, 258 c2, 407 c1, 407 c2, 407 c3, 427 c1, 432 c1, 432 c2, 432 c3) diallel crosses including selfs without reciprocals were made. With both these diallel sets of crosses not all possible $\frac{1}{2}$ n (n + 1) combinations were made. Plants of the populations thus obtained were planted in the field in a completely randomized block design with 20 blocks (of each population one plant per block). At the end of the growing period stem length was measured. Combining ability analyses of variance on these figures were made, followed by comparisons of means and variances of sum-populations. A sum-population is a collection of similar populations.

The following types of sum-populations were distinguished:
sum-population c⊗, this is a collection of c⊗ populations obtained by selfing of c plants from one parent.
sum-population c × c, this is a collection of c × c populations obtained by crosses between c plants from one parent or between c plants from different parents.
sum-population m⊗, this is a collection of m⊗ populations obtained by selfing of m plants from one female parent.
sum-population m × m, this is a collection of m × m populations obtained by crosses between m plants from one female parent or between m plants from different female parents.

Comparisons of g.c.a.'s of matromorphic and control plants for stem length and variance of stem length. After prickle pollination of one clone of var. *gemmifera* (DC.) SCHULZ cv. Hybride 69002, 18 matromorphic plants were obtained. From the same clone 18 control plants were obtained after selfing. Diallel crosses, including selfs, without reciprocals were made between the matromorphic plants and also between the control plants. With both diallel sets of crosses not all possible $\frac{1}{2}$ n (n + 1) combinations were made. Plants of the populations thus obtained were planted in the field in a completely randomized block trial with 20 blocks (of each population one plant per block). At the end of the growing period stem length was measured and mean stem length and variance of stem length of each population were calculated. Combining ability analyses of variance on these figures were made.

Stem length and head weight were chosen for these investigations because they could easily be measured with great precision.

The combining ability analyses of variance required for the model $y_{ij} = \mu + \lambda_i + \lambda_j + s_{ij} + e_{ij}$ were made according to the general method for complete and incomplete diallel crosses proposed by GARRETSEN & KEULS (1973).

In this model

μ = a general mean level.

$\lambda_i(\lambda_j)$ = the general combining ability of the $i^{th}(j^{th})$ parent.

s_{ij} = the specific combining ability for the cross between the i^{th} and j^{th} parents.

e_{ij} = a random error.

Tests for significance were made for means with the T-test and for variances with the F-test.

Variance for stem length or head weight is always taken to mean the variance for plants. By population is meant a group of plants.

RESULTS AND DISCUSSION

Comparisons of means and variances of stem length and head weight of matromorphic and control populations. Table 2 shows the means and variances of stem length and head weight of matromorphic and control populations. As seen in this table the means for stem length or head weight of matromorphic populations are greater than or equal to the means for these characters of populations obtained from the same parent by selfing. For Kolos (1971) this difference is significant. The means for stem length or head weight of the female parent population exceeded the means for these characters of the two other types of populations (significant differences for Hybride 69002 (1971) and Putjes) with the exception of the mean for head weight of the female parent population Kolos. The greater head weight of the matromorphic population, compared with the head weight of the female parent population, must be attributed to accidental factors.

Significant differences between matromorphic and control populations also occur for variances of these characters. For instance the variance for head weight of the matromorphic population of Kolos (1971) is significantly greater than this variance for the female parent ⊗ population. The difference for variance of stem length between the matromorphic and the female parent population of Hybride 69002 (1971, 1972) is also significant.

If means for quantitative characters of matromorphic populations are significantly smaller than such means of the female parent populations or if variances of matromorphic and female parent populations are significantly different then genetic recombination must have occurred (see Table 1). Therefore from these results it is not likely that (many) matromorphic embryos have developed from egg-cells which were diploid as a result of pre-meiotic endomitosis and autobivalent formation at meiosis or as a result of absence of meiosis. From the mean values of the investigated quantitative characters of the matromorphic and control populations it may further be concluded that matromorphic embryos not (often) developed from egg-cells which were diploid as a result of chromosome doubling during or after gametogenesis. It is

Table 2. Means and variances of stem length (cm) and head weight (kg) of matromorphic and control popula

Female parent	Character	Population	Number of plants	
			1971	1972
B. oleracea var. *capitata* cv. Kolos	head weight	matromorphic	14	23
		female parent ⊗	25	35
		female parent	11	
B. oleracea var. *capitata* cv. Roem van Enkhuizen	head weight	matromorphic	9	
		female parent ⊗	19	
		female parent	20	
B. oleracea var. *gemmifera* cv. Hybride 69002	stem length	matromorphic	65	20
		female parent ⊗	58	29
		female parent	27	30
B. oleracea var. *sabauda* cv. Hammer	head weight	matromorphic	5	10
		female parent ⊗		24
		female parent	43	
B. oleracea var. *sabauda* cv. Putjes	head weight	matromorphic	10	
		female parent	21	

Equal letters (a or b) for means or variances, per parent per year indicate no significant difference. Unequal letters indicate significant differences.

more probable that deviations in cell or nuclear divisions during meiosis were responsible for the formation of diploid products of gametogenesis.

As d and h values of the characters investigated are not known and diploid products of gametogenesis may develop in different ways, it is not possible to say more about the formation of matromorphic embryos on the basis of these figures.

Comparisons of means and variances of stem length of sum-populations obtained from diallel crosses between matromorphic plants and between control plants. In analysing differences in means for stem length of populations obtained from diallel crosses between 11 matromorphic plants and diallel crosses between 9 control plants, it is of interest in view of the occurrence of inbreeding depression or heterosis, to know what part of the total genetical variation can be ascribed to variation due to general combining ability (g.c.a.), mainly caused by additive gene action and what part to variation due to specific combining ability (s.c.a.), mainly caused by non-additive gene action. Combining ability analyses of variance were made to investigate this.

From Table 3 it appears that both significant g.c.a.'s and s.c.a.'s for stem length occur for matromorphic and control plants. This was also found for stem length of *Brassica juncea* plants by SINGH (1973). For matromorphic and for control plants the differences between σ^2 g.c.a and σ^2 s.c.a. are small, meaning that additive and non-additive effects are both important (if non-allelic interactions are of minor importance). This implies that in case of heterozygosity of matromorphic plants their progenies obtained by selfing may show considerable inbreeding depression. If matromorphic plants are more or less heterozygous than control plants, heterosis of progenies obtained from diallel crosses between matromorphic plants and between control plants respectively, may be different. Table 4 shows differences in stem length between sum-populations. The means for stem length of the m⊗ sum-

tions.

Mean		Variance		Coefficient of variation	
1971	1972	1971	1972	1971	1972
2.29a	2.05a	0.81a	1.48a	39.3	59.2
1.57b	1.88a	0.30b	1.13a	35.0	56.5
1.89ab		0.36ab		31.7	
2.44ab		0.25a		20.6	
2.21a		0.28a		24.1	
2.93b		0.82b		30.9	
65.7a	50.1ab	88.2a	109.3a	14.3	20.9
66.1a	47.8b	84.1a	92.8a	13.9	20.2
70.7b	53.1a	30.1b	40.9b	7.8	12.1
2.31a	3.80a	0.65a	0.99a	34.8	26.1
	3.77a		0.85a		24.4
2.93b		0.90a		32.3	
1.16a		0.80a		77.1	
2.04b		0.48a		34.0	

populations were smaller than these means of the corresponding c populations (no figures for hybrid 427). This indicates that inbreeding depression has occurred after selfing of the matromorphic plants. For in an earlier experiment (see Table 5) the means for stem length of the matromorphic plants appeared to be never smaller (except for hybrid 427) than the mean stem length of the corresponding control plants. This can also be seen in Table 2 for matromorphic and control populations from other (female) parents.

Thus matromorphic plants appear to be heterozygous for stem length. The mean stem lengths of the c $\times$ c and m $\times$ m sum-populations were in most cases significantly greater than those of the c$\otimes$ and m$\otimes$ sum-populations (see Table 4). Differences between m$\otimes$ and c$\otimes$ sum-populations were small and not significant. Various significant differences for mean stem lengths occur between c $\times$ c^w and m $\times$ m^w sum-populations and between c $\times$ c^b and m $\times$ m^b sum-populations. The means of the m $\times$ m sum-populations were in almost all cases greater than the means of the cor-

Table 3. Combining ability analyses of variance for stem length and estimates of the variance components.

Source of variation	Matromorphic plants			Control plants		
	degrees of freedom	mean squares	Estimates of variance components	degrees of freedom	mean squares	Estimates of variance components
g.c.a.	10	120.50^{++}	8.90	8	48.21^{++}	4.34
s.c.a.	50	13.76^{++}	9.50	28	9.18^{++}	5.37
error	1002	4.26	4.26	586	3.81	3.81

Table 4. Differences between sum-populations for mean stem length (cm).
c = control plant (female parent ⊗), m = matromorphic plant, w = crosses within a population (between m or between c plants from one (female) parent), b = crosses between populations (between m or between c plants from different parents).

Female parent	(Sum-) population	Number of populations	Number of plants	Mean c	c⊗	c × c^w	c × c^b	m⊗	m × m^w	m × m^b
258	c	1	20	68.5	7.2$^+$		0.3	5.6		− 5.8$^+$
	c⊗	2	27	61.3			−6.9^{++}	−1.6		−13.0^{++}
	c × c^b	13	212	68.2				−5.3^{++}		− 6.1^{++}
	m⊗	1	19	62.9						−11.4^{++}
	m × m^b	16	240	74.3						
407	c	1	19	71.1	7.5$^+$	6.3$^+$	3.5	4.6	−2.2	− 0.9
	c⊗	2	28	63.6		−1.2	−4.0	−2.9	−9.7^{++}	− 8.4^{++}
	c × c^w	2	22	64.8			−2.8	−1.7	−8.5$^+$	− 7.2^{++}
	c × c^b	15	250	67.6				1.1	−5.7$^+$	− 4.4^{++}
	m⊗	3	19	66.5					−6.8$^+$	− 5.5^{++}
	m × m^w	3	27	73.3						1.3^{++}
	m × m^b	22	370	72.0						
427	c	1	20	67.2	9.2$^+$		−0.2			− 3.8
	c⊗	2	42	58.0			−9.4^{++}			−13.0^{++}
	c × c^b	8	145	67.4						− 3.6^{++}
	m × m^b	10	155	71.0						
432	c	1	21	67.2	−2.3	−5.0	−2.4	0.9	−3.8	− 5.8$^+$
	c⊗	3	59	69.5		−2.7	−0.1	3.2	−2.5	− 3.5^{++}
	c × c^w	3	55	72.2			2.6$^+$	5.9^{++}	1.2	− 0.8
	c × c^b	14	253	69.6				3.3$^+$	−1.4$^+$	− 3.4^{++}
	m⊗	5	54	66.3					−4.7^{++}	− 6.7^{++}
	m × m^w	10	205	71.0						− 2.0^{++}
	m × m^b	29	588	73.0						

Table 5. Means for stem length of matromorphic and control plants, 85 days after sowing.

Female parent	Type of plants	Number of plants	Mean stem length (cm)
258	matromorphic	3	16.5
	female parent ⊗	10	14.1
407	matromorphic	3	11.7
	female parent ⊗	10	11.6
427	matromorphic	1	9.5
	female parent ⊗	11	13.2
432	matromorphic	5	12.8
	female parent ⊗	11	10.6

responding c × c sum-populations. This may indicate that the genotypes for stem length of matromorphic and control plants differ essentially.

Significant differences also occur in variances of different m⊗ sum-populations and for variances of m⊗ and c⊗ sum-populations.

These results suggest that the matromorphic plants had different degrees of hete-

rozygosity for stem length, probably caused by different ways of origination. Completely homozygous plants did not seem to occur.

Comparisons of g.c.a.'s of matromorphic and control plants for stem length and variance of stem length. Combining ability analyses of variance were made on figures for stem length of populations obtained from diallel crosses, selfs included, between 18 matromorphic plants and between 18 control plants. Results are presented in Table 6. As shown in this table, for matromorphic plants both significant g.c.a.'s and significant s.c.a.'s for stem length are present. For control plants only significant g.c.a.'s occur. The difference between σ^2 g.c.a. and σ^2 s.c.a. for matromorphic plants is small, meaning that additive and non-additive effects may be about equally important. With both matromorphic and control plants significant g.c.a.'s for variance of stem length were also present.

G.c.a.'s for stem length and for variance of stem length of matromorphic and control plants are shown in Table 7. As can be seen in this table the range for g.c.a.'s for stem length was about the same for matromorphic and control plants, in contrast with the range for g.c.a.'s for variance of stem length. This range was greater for the matromorphic than for the control plants.

The correlation between g.c.a.'s for stem length and g.c.a's for variance of stem length of matromorphic and control plants was investigated. This correlation is highly significant and positive for the control plants (correlation coefficient = 0.84) but not significant for the matromorphic plants (correlation coefficient = 0.34). Fig. 1 illustrates these differences in correlation.

The difference in correlation of g.c.a.'s for stem length and g.c.a.'s for variance of stem length with matromorphic and control plants, respectively, may be caused by essential differences in heterozygosity of genotypes for stem length of these plants. From the ranges of g.c.a.'s for variance of stem length it may be concluded that some matromorphic plants occurred with very heterozygous genotypes for stem length and some matromorphic plants with relatively homozygous genotypes for this character. The level of heterozygosity of matromorphic plants is related to the time of chromosome doubling resulting in diploid products of gametogenesis from which matromorphic embryos may develop (EENINK, 1974).

From the comparisons of g.c.a.'s for variance it appears that matromorphic plants developed from egg-cells which were diploid as a result of deviations in cell or nuclear divisions at different moments. Plants 35.95 and 54.130 (see Table 7) for instance

Table 6. Combining ability analyses of variance for stem length and estimates of the variance components.

Source of variation	Matromorphic plants			Control plants		
	degrees of freedom	mean squares	estimates of variance components	degrees of freedom	mean squares	estimates of variance components
g.c.a.	17	62.29++	3.71	17	123.84++	8.09
s.c.a.	108	6.67++	3.64	105	0.74	0
error	∞	3.03	3.03	∞	2.50	2.50

Table 7. G.c.a.'s for stem length (cm) and variance of stem length in populations obtained from diallel crosses between matromorphic plants and between control plants. m = mean.

Matromorphic plants	G.c.a.'s		Control plants	G.c.a.'s	
	stem length (m = 40.36)	variance (m = 45.39)		stem length (m = 39.12)	variance (m = 38.99)
35.95	+2.94	+23.50	52.50	−1.73	− 5.51
37.56	+0.62	− 6.41	52.57	+5.96	+15.82
37.166	+1.41	− 6.10	52.62	−5.30	−13.74
38.37	−0.34	− 9.19	52.76	−1.31	− 1.97
38.120	+1.26	− 1.62	52.85	+1.38	+ 3.82
38.158	+2.73	− 3.55	52.94	−2.78	− 7.93
39.68	−1.05	+ 5.06	52.102	−0.98	+ 2.94
39.143	−3.14	−13.04	52.111	−0.90	+ 2.57
42.119	+3.10	−14.94	52.126	−0.36	− 2.35
45.115	−4.29	− 0.86	52.138	−1.65	− 4.85
47.45	−0.22	+10.08	52.145	−0.39	− 4.39
47.146	−1.73	− 9.14	52.156	+0.94	+ 6.87
50.182	−1.03	+ 1.25	52.170	−1.23	− 3.23
54.52	+0.53	+11.67	52.177	+1.09	+ 4.88
54.130	+0.70	+19.37	52.183	+2.77	− 4.37
55.4	−2.62	− 5.43	53.128	+4.72	+ 5.30
55.155	−1.62	−13.94	53.151	−3.35	− 8.37
66.180	+2.71	+13.36	53.184	+3.14	+14.51

could have been developed from diploid egg-cells resulting from absence of meiosis or from a tetraploid EMC (pre-meiotic endomitosis) and autobivalent formation, on account of the heterozygosity of the genotype for stem length. Plants 39.143, 42.119 and 55.155 may be rather homozygous and could have been developed from egg-cells which were diploid as a result of a deviation in the second meiotic division. The other matromorphic plants may have been developed from egg-cells which were diploid as a result of deviations in the first meiotic division.

Of 17 matromorphic plants, investigated for their g.c.a.'s for variance of stem length, the genotype for incompatibility was also known. These g.c.a.'s and S-genotypes were compared as shown in Table 8. By using the rank test of Wilcoxon (MANN & WHITNEY, 1947) it could be demonstrated that no random distribution of g.c.a.'s for variance over S-genotypes occurred. Plants with a homozygous S-genotype (SxSx or SySy) often had smaller values for g.c.a.'s for variance than plants with a heterozygous S-genotype (SxSy). Thus a linkage appears to exist between the incompatibility gene and (a) gene(s) for stem length. This implies that (qualitative) research on S-genotypes may give information on the level of heterozygosity of a quantitative character (stem length) of the matromorphic plants.

CONCLUSIONS

Comparisons of means and variances for stem length and head weight of matromorphic and control populations or their progeny and comparisons of g.c.a.'s for stem length and variance of stem length of matromorphic and control plants show that

Table 8. Distribution of g.c.a.'s for variance of stem length over S-genotypes of matromorphic plants.

G.c.a.'s	
SxSx or SySy	SxSy
−14.94	
−13.94	
−13.04	
− 9.19	
	− 9.14
− 6.41	
− 6.10	
− 5.43	
− 3.55	
	− 1.62
	+ 1.25
+ 5.06	
+10.08	
	+11.67
	+13.36
	+19.37
	+23.50

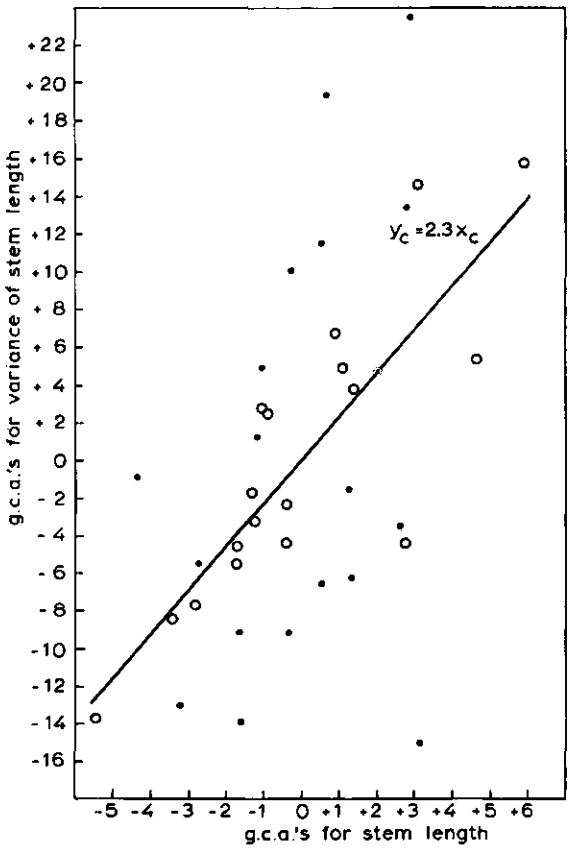

Fig. 1. G.c.a.'s for stem length and variance of stem length. ● = matromorphic plants (m), ○ = control plants (c).

matromorphic plants were heterozygous. Progenies of matromorphic plants obtained by selfing show inbreeding depression. Significant differences in the level of heterozygosity of matromorphic plants point to different ways of origination of diploid products of gametogenesis. This agrees with conclusions mentioned in a previous paper (EENINK, 1974).

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MATROMORPHY IN *BRASSICA OLERACEA* L. VI. RESEARCH ON OVULES, EMBRYOS AND ENDOSPERMS AFTER PRICKLE POLLINATION

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SUMMARY

A research has been carried out on possible differences in developmental rate or growth rate of ovules, embryos and endosperms after prickle pollination and after selfing of *Brassica oleracea* plants. After prickle pollination ovules, embryos and endosperms developed/grew slower than after selfing which may be attributed to certain disturbances in the embryosac. After prickle pollination embryos and endosperms occurred with possible a rather high ploidy level suggesting that diploid embryosacs were present.

INTRODUCTION

Deviations in cell or nuclear divisions result in diploid products of gametogenesis from which matromorphic embryos can develop. Probably these deviations occur before or during sporogenesis (EENINK, 1974b, 1974c, 1974d) and embryosacs of diploid *Brassica oleracea* plants will then be diploid. After prickle pollination, diploid (matromorphic) or triploid and tetraploid (hybrid) embryos may develop from egg-cells in such embryosacs. The tetraploid embryos result from fusion of unreduced female and male gametes. Endosperm formation may not be necessary for the development of apomictic embryos as for instance with *Orchis* (RUTISHAUSER, 1969) and *Populus* (STETTLER & BAWA, 1971) though normally as e.g. in *Triticum* (KATAYAMA, 1933; KIHARA, 1940) and in *Solanum* (VON WANGENHEIM et al., 1960), endosperm development is of vital importance. If after prickle pollination of *B. oleracea* plants with diploid embryosacs, endosperm formation should occur, then the primary endosperm nucleus may be tetraploid (autonomous development), pentaploid or hexaploid (development after fertilization). This is shown in Table 1.

If deviations occur in nuclear divisions during gametogenesis (RUTISHAUSER, 1967) the embryosacs may contain nuclei with different ploidy levels (haploid and diploid). If deviations occur after gametogenesis, the embryosacs are haploid. Depending on the moment on which these deviations occur, after prickle pollination haploid, diploid triploid or tetraploid embryos may develop with e.g. diploid, triploid or tetraploid endosperms (see Table 1). A number of these embryos may develop parthenogenetically, others are hybrids.

The quantitative and/or qualitative genomic differences mentioned above within embryosacs of ovules after prickle pollination may explain possible differences in rate of development and growth of ovules, embryos and endosperms after prickle pollination and selfing respectively (WATKINS, 1932; MÜNTZING, 1933; HOWARD, 1939, 1942, 1947; COOPER & BRINK, 1940; THOMPSON & JOHNSTON, 1945; BRINK &

Table 1. Ploidy levels of nuclei in unreduced and reduced embryosacs. A = genome of the female parent; B = genome of the male parent.

Fertilization	Diploid embryosac		Haploid embryosac	
	embryo nuclei	primary endosperm nucleus	embryo nuclei	primary endosperm nucleus
no fertilization	2A	4A	A	2A
single fertilization				
generative nuclei: fused/unreduced	2A	4A + 2B	A	2A + 2B
	2A + 2B	4A	A + 2B	2A
not fused/reduced	2A	4A + B	A	2A + B
	2A + B	4A	A + B	2A
double fertilization				
generative nuclei: unreduced	2A + 2B	4A + 2B	A + 2B	2A + 2B
reduced	2A + B	4A + B	A + B	2A + B

COOPER, 1947; HAKANSSON, 1953; SKIEBE, 1958, 1973; VON WANGENHEIM, 1961; RUTISHAUSER, 1969; NEUMANN, 1973).

This paper reviews the results of research on ovules, embryos and endosperms after prickle pollination or selfing. The investigations were made to obtain more information on the moment of chromosome doubling, resulting in the formation of diploid (matromorphic) embryos, and on the way these embryos and endosperms develop after prickle pollination.

MATERIALS AND METHODS

Plants of a clone of *Brassica oleracea* L. var. *capitata* L. cv. Kolos grown at 14°C in a phytotron were prickle pollinated by plants of *Raphanus sativus* L. var. *radicula* PERS. cv. Huizer's Triplo. This parental combination was used because the variety Kolos never formed interspecific or intergeneric hybrids in crosses made before (EENINK, 1974a). Therefore the developing embryos to be investigated here will likely be matromorphics. Some results mentioned in this paper were obtained from interspecific or intergeneric crosses (involving *Brassica*) different from the one used here.

Pistils or siliques were collected between 0 and 60 days after prickle pollination and fixed in Carnoy-solution. They were embedded in paraffin using alcohol and xylol (alcohol 35%, 50%, 70%, 85%, 96%; xylol; paraffin). In addition microtome sections were cut (18 μm) which were stained as follows (according to a modified method of FLEMMING (1891) and CONN (1940), KROON unpublished); two times xylol (each time 6–10 min), alcohol 96% (15 min), picric acid in alcohol 96% (1 min), rinsed in water (1 min), alcohol 35% (1 min), 1% safranin in alcohol 50% (2 h), rinsed in water (1 min), IKI in alcohol 80% (0.5 min), rinsed in water (1 min), 1% crystal violet in water (1 h), rinsed in water. Finally colour differentiation of the sections was done in cuvettes filled with; IKI, alcohol 96%, picric acid, alcohol 96%, alcohol

100%, oil of cloves (eugenol) and xylol respectively. The sections were mounted in balsem.

The volume of the ovules functioned as a criterium for the growth of the ovules after prickle pollination and selfing respectively. The volumes were calculated from length, width, shape and thickness (number of sections × 18 μm) of the ovules. The developmental stages of embryos were classified according to a system of WARDLAW (1955). In order to get an impression of possible differences in ploidy levels of embryos or endosperms, diameters of nuclei were measured; chromosomes were not counted because very few dividing nuclei could be observed. Of each embryo or endosperm 10 nuclei were measured and means for these figures were calculated.

RESULTS AND DISCUSSION

Development and growth

Ovules. Observation of ovules revealed that both normal and abnormal (shrunken) ovules occurred. From Fig. 1 it appears that normal ovules grew slightly slower after prickle pollination than after selfing. The volumes of these ovules varied considerably, in particular after prickle pollination. The abnormal ovules correspond with deviating seeds, which were often smaller than normal seeds and were shrunken or

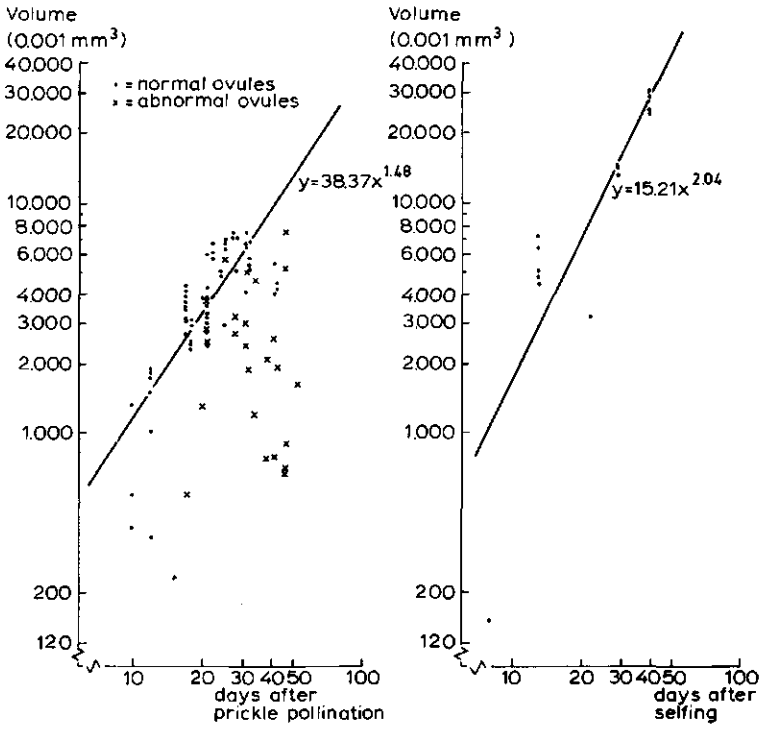

Fig. 1. Growth of ovules after prickle pollination or selfing.

Table 2. Length (mm) of 44 matromorphic and 246 hybrid seeds obtained after prickle pollination of various botanic varieties of *Brassica oleracea*.

Type of seeds	Distribution (%) of seeds over lengths																				Mean	Variance
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
Hybrid seeds	1	0	2	7	12	24	17	20	10	5	0	1									15.8	3.3
Matromorphic seeds											2	18	20	7	7	7	9	14	14	2	24.2	7.4

collapsed. In the abnormal ovules sometimes no embryos or deviating embryos or endosperms were present. The abnormal ovules or seeds were often formed after prickle pollination (15–20% of the investigated ovules) and very rarely after selfing. Abnormal ovules in *Brassica*, after interspecific or intergeneric crosses have also been found by NISHI & HIRAOKA (1962). Shrunken seeds from parental combinations, different from the one used here, were placed on a nutrient medium (as used by GUHA & MAHESHWARI, 1964). A number of these seeds germinated and were found to contain hybrid embryos. This has also been found for such seeds from many other interspecific or intergeneric crosses.

Mature seeds of botanic varieties of *B. oleracea* could be distinguished by their size as matromorphic or hybrid seeds as is shown in Table 2. Matromorphic seeds were significantly larger than hybrid seeds. This was also found by NISHI et al. (1964), TOKUMASU (1965) and HEYN (1973). HOWARD (1939, 1942, 1947) also found that, after interspecific crosses with *Brassica* and *Nasturtium*, respectively, hybrid seeds were relatively small. The fact that hybrid seeds were rather small may be caused by disturbances in development or growth as a result of qualitative and/or quantitative genomic differences between embryo, endosperm and female parent tissue or differences between genomes within embryos and endosperms (RUTISHAUSER, 1969). The matromorphic seeds were often larger than seeds obtained by selfing of the same parent. This may be due to the fact that after prickle pollination one or two seeds per silique developed whereas after selfing or crossing more than 20 seeds were formed. Therefore the matromorphic seeds may have had more nutrients available than the seeds from selfing or crossing.

Embryos. Brassica embryos develop according to the Onagraceae type (Crucifer type; RUTISHAUSER, 1969). In the present experiments the first embryo (two cells) was found eight days after prickle pollination. THOMPSON (1953) and NISHI & HIRAOKA (1962) found *Brassica* embryos consisting of five and three cells respectively, five days after (prickle) pollination. According to HARBERD (1969) many embryos, cultured in vitro, had passed through the globular phase at 7 days after pollination. We observed that *Brassica* embryos developed slower after prickle pollination than after selfing. For instance phase III (heart phase) was already found 13 days after selfing but not before 21 days after prickle pollination (see Fig. 2 and 3). Embryos of a certain age, however, show a great variation in developmental stages, both after prickle pollination and after selfing. For instance 42 days after prickle pollination still phase II (reversed trapezium) was found incidentally which points to a delayed embryo development.

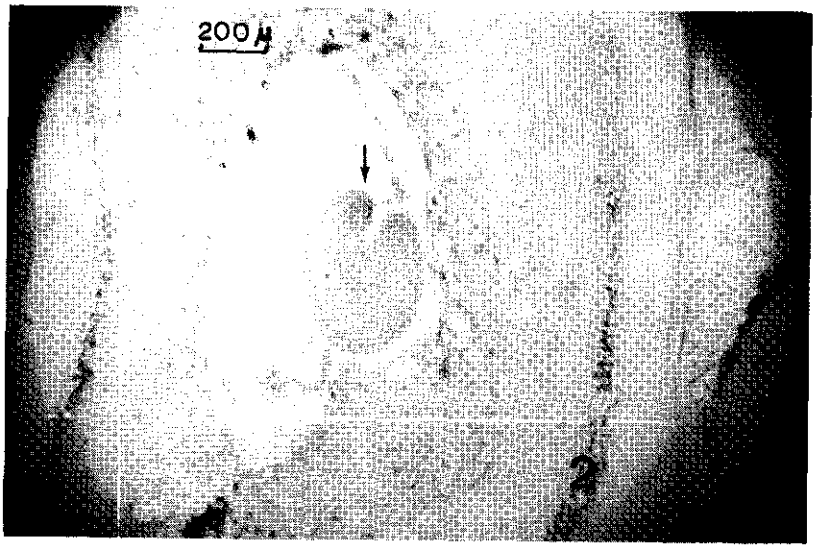

Fig. 2. Embryo in developmental phase III, 13 days after selfing.

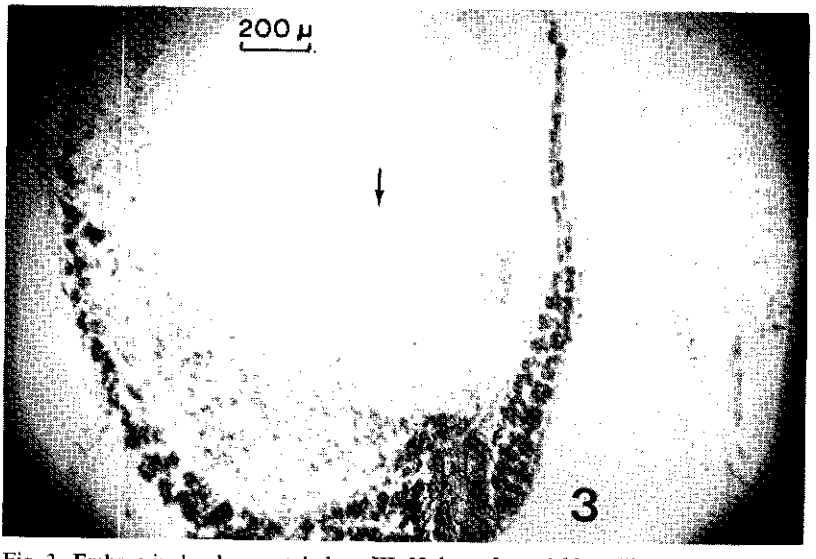

Fig. 3. Embryo in developmental phase III, 33 days after prickle pollination.

The embryos in the abnormal (shrunken) ovules also show a large variation in developmental phase. Despite the degenerated habit of such ovules, 45 days after prickle pollination embryos were found with developmental phase V (torpedo) though also embryos of the same age occurred with developmental phase I (globular) (see Fig. 4 and 5).

The size of embryos formed after prickle pollination or selfing at corresponding

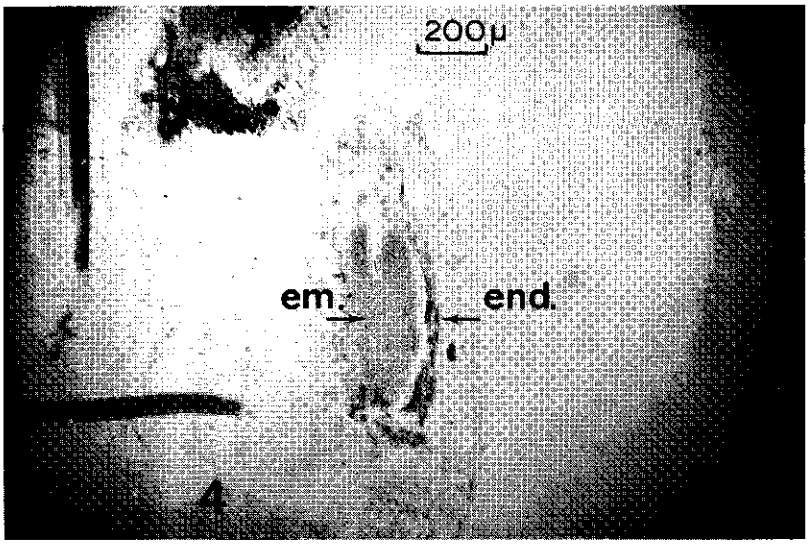

Fig. 4. Embryo (em.) in developmental phase IV and endosperm (end.) in an abnormal ovule, 41 days after prickle pollination.

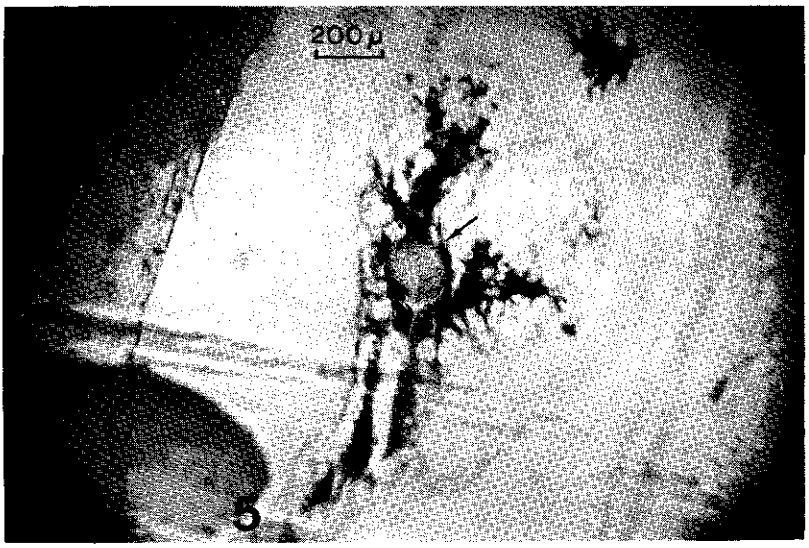

Fig. 5. Embryo in developmental phase I in an abnormal ovule, 28 days after prickle pollination.

ages and developmental phases, often differed significantly. The matromorphic embryos were often smaller than those formed after selfing. These differences in size, possibly correspond with differences in growth rate (plant height, etc.) in the early phases of development between matromorphic plants and I1 plants (obtained by selfing) from the same parent. Until about 35–45 days after germination matromorphic plants were usually smaller than the I1 plants as shown in Table 3. After

Table 3. Mean plant length (cm) of a number (n) of matromorphic (m) and I_1 plants (i) of various botanic varieties of *Brassica oleracea* at 14°C.

Days after sowing	Var. *capitata* cv. Kolos		Var. *gemmifera* cv. experimental hybrid		Var. *gemmifera* cv. Hybride 69002		Var. *sabauda* cv. Hammer	
	m(n=23)	i(n=35)	m(n=5)	i(n=10)	m(n=20)	i(n=29)	m(n=10)	i(n=24)
13			13.0	15.1				
20			13.0	15.4				
24			15.2	16.5				
27			19.8	22.4				
30	14.2	15.5			12.5	12.2	11.7	17.2
42			46.8	45.0				
85			128.0	106.3				
136					70.6	67.1		

45 days the size of the matromorphic plants was equal to or larger than the size of the I1 plants (EENINK, 1974d).

Endosperms. Both after prickle pollination and after selfing endosperm developed. After prickle pollination, however, the endosperm grew slower than after selfing, which agrees with results from interspecific crosses with *Brassica* made by NISHI & HIRAOKA (1962). Endosperm was also present in abnormal ovules, but was then generally smaller than endosperm in normal ovules after prickle pollination. The endosperm in the abnormal ovules was often lobed and granular and was mainly concentrated around the embryo. The deviating endosperm may have developed autonomously, as e.g. in *Hypericum* (NOACK, 1939) and *Arabis* (BÖCHER, 1951).

Ploidy levels

Embryos. In Fig. 6 the diameters of nuclei of embryos developed after prickle pollination and after selfing are shown. From this figure it appears that no significant differences occurred between the means for embryos formed after prickle pollination and selfing respectively. This may indicate that both groups of nuclei had the same ploidy level and were diploid. However, the variation in nuclear diameters of embryos formed after prickle pollination was greater than that for embryos arisen after selfing. This may be due to the presence of, perhaps, hybrid embryos with triploid or tetraploid nuclei. The nuclei of embryos in abnormal ovules after prickle pollination were not significantly smaller than those of embryos in normal ovules after prickle pollination. This may imply that these two types of ovules had embryos with the same ploidy level though in some abnormal ovules haploid embryos may have occurred.

Endosperms. Fig. 7 shows the mean diameters of endosperm nuclei in embryosacs after prickle pollination and after selfing. From this figure it appears that mean nuclear diameters of the two groups of endosperms did not differ significantly. The variation in mean nuclear diameters was greater after prickle pollination than after selfing. After prickle pollination endosperms with very large nuclei occurred. They

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MATROMORPHY IN *BRASSICA OLERACEA* L. VII. RESEARCH ON PRODUCTS OF MICROSPOROGENESIS AND GAMETOGENESIS FROM PRICKLE POLLINATED PLANTS

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SUMMARY

A research has been carried out on the occurrence of 2n gametes in *Brassica oleracea* and on their possible way of origination. After microsporogenesis it appeared that unreduced PMC's, dyads and triads occurred. Giant pollen grains with two (diploid) and four (haploid) generative nuclei, respectively, were found, resulting from a deviating cytokinesis and karyokinesis. Diploid heterozygous matromorphic embryos may develop from such unreduced gametes formed after macrosporogenesis.

INTRODUCTION

Matromorphic embryos from diploid *Brassica* plants probably originate from diploid egg-cells (TOKUMASU, 1965; HODGKIN & REDFERN, 1971; MACKAY, 1972; EENINK, 1974b, 1974c, 1974d). TOKUMASU (1965) suggested that these diploid egg-cells developed from EMC's which were tetraploid as a result of pre-meiotic endomitosis. According to HODGKIN & REDFERN (1971), MACKAY (1972) and EENINK (1974c, 1974d) disturbances of meiosis lead to the formation of unreduced egg-cells.

After interspecific or intergeneric crosses, many research workers found triploid or tetraploid hybrids resulting from the occurrence of unreduced gametes in *Brassica* plants (TERASAWA, 1932; KARPECHENKO, 1937a, 1937b; MORINAGA & KURIYAMA, 1937; RAMANUJAM, 1940; RUDORF, 1951; OLSSON et al., 1955; JAHR, 1962a, 1962b; HUZIWARA et al., 1965; HEYN, 1973). Interspecific or intergeneric hybrids, involving *Brassica* also often produced unreduced (2n) gametes (KARPECHENKO, 1924, 1927, 1928, 1937a, 1937b; U et al., 1937; RAMANUJAM & SRINIVASACHAR, 1943; RUDORF, 1951; HOFFMANN & PETERS, 1958; JAHR, 1962a, 1962b; HEYN, 1973). In many other genera, such as *Solanum* (HANNEMAN & PELOQUIN, 1967, 1968) and *Datura*, *Poa*, *Potentilla* and *Taraxacum* (RUTISHAUSER, 1967) unreduced gametes were also found.

Investigations have been carried out on the moment or cause of disturbances of nuclear or cell divisions resulting in the formation of unreduced gametes in *Brassica*. MORINAGA (1929b) and FUKUSHIMA (1930) e.g. found PMC's in *Brassica* plants which were possibly tetraploid as a result of deviations in nuclear divisions before meiosis. Pre-meiotic endomitosis in *Brassica* was also found by IIZUKA (1961). MORINAGA (1929a), U (1935) and MORINAGA & KURIYAMA (1937) stated that deviations, like fusion of spindles, occurred during meiosis. Sometimes disturbances of chromosome pairing were thought to be responsible for the occurrence of deviating products of sporogenesis (TERASAWA, 1928; MORINAGA, 1929a, 1929b, 1934; KARPECHENKO,

1927, 1928, 1937a, 1937b; U, 1935; SIKKA, 1940; RAMANUJAM & SRINIVASACHAR, 1943; IIZUKA, 1961). Such disturbances were also responsible for the formation of dyads in e.g. *Camellia* (KATO & SIMURA, 1970). In *Datura* the second meiotic division was found not to occur (SATINA & BLAKESLEE, 1935). With *Solanum* also rather extensive research has been carried out on the occurrence of unreduced gametes. PRAKKEN & SWAMINATHAN (1952) suggested that unreduced gametes may result from premeiotic, meiotic (first and second meiotic division) and post-meiotic disturbances. OPPENHEIMER (1933), IVANOVSKAJA (1941), HÖGLUND (1970) and RAMANNA (1974) stated that fusion of second metaphase spindles may lead to the formation of unreduced gametes. KOOPMANS & VAN DER BURG (1951) supposed that doubling of chromosomes took place after pollination. The frequency of 2n gametes possibly is determined genetically as was found in *Brassica* (IIZUKA, 1961) and *Primula* (SKIEBE, 1969, 1972), though the environment may also play an important role (RUTISHAUSER, 1967).

In view of the diploid character of the matromorphic plants it is of interest to know if they originate from diploid, unreduced, gametes. Therefore a research has been carried out on the occurrence of unreduced gametes in prickle pollinated *Brassica* plants and on their possible way of origination. Besides the correlation between the occurrence of matromorphy and unreduced gametes was investigated.

MATERIALS AND METHODS

Because macrosporogenesis and its resulting products are rather difficult to study, the resulting products of microsporogenesis were investigated, though it is not absolutely certain whether deviating processes in microsporogenesis also occur (and in the same frequency) in macrosporogenesis.

Research on products of microsporogenesis. Very young anthers from buds (1–2 mm) of plants of *Brassica oleracea* L. var. *capitata* L. cv. Kolos, grown at 14°C in a phytotron were collected and fixed in Carnoy-solution. The anthers were then macerated and stained in acetic orcein. Products directly resulting from sporogenesis (tetrad phase) were investigated.

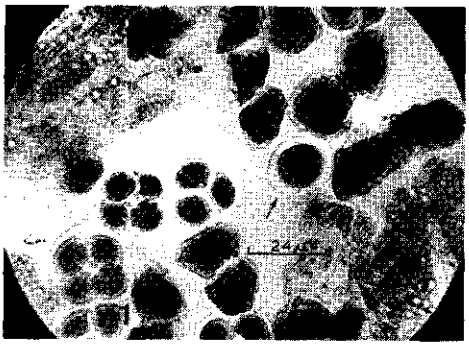
Research on pollen grains. Mature pollen from plants of the variety Kolos, mentioned above, was collected and stained with lactophenol acid fuchsin. Pollen was examined for the presence of deviating, giant, pollen grains resulting from disturbances before or during meiosis. Lactophenol acid fuchsin gives the pollen grains a bright red appearance. They were measured by using a measuring ocular (one unit of the scale = 3 μm). Pollen grains were considered to be deviating if their length was equal to or greater than 10 units on the scale of the measuring ocular on the basis of preliminary observations.

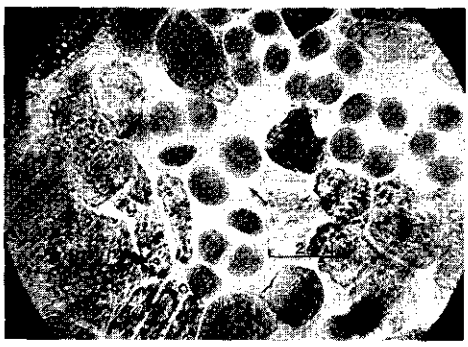
Pollen from the same Kolos plants was collected and stained with chloral carmine. The pollen grains take on a light-red orange colour, the nuclei a more red colour. The number and the size of the generative nuclei in the giant pollen grains were studied. The vegetative nuclei very rarely become stained. Perhaps they are degenerated.

Research on a correlation between the occurrence of giant pollen grains and matromorphic seeds. Of 11 clones of the variety Kolos, investigated for their individual parthenogenetic ability (i.p.a.; EENINK, 1974a), the frequency of giant pollen grains (percentage of the number of pollen grains investigated) per clone was determined. Of each clone 50,000–60,000 pollen grains from various plants were screened after staining with lactophenol fuchsin acid. The correlation between the percentage of giant pollen grains and the i.p.a. was investigated.

RESULTS AND DISCUSSION

Research on products of microsporogenesis. From the investigated products of sporogenesis it appeared that different deviating types occurred in different frequencies. Unreduced PMC's were predominant (see Fig. 1). Dyads and triads also occurred (see Fig. 2 and 3). The dyads result either from failure of reductional cell wall formation (one equational wall is formed) or from failure of both equational cell walls to form. The triads may result from a partially formed reductional cell wall or from failure of the formation of one of both equational walls (RAMANNA, 1974). Because cytokinesis may be more or less independent of karyokinesis (RAMANNA, 1974) these deviating products of sporogenesis need not necessarily be unreduced. Some of them may contain diploid nuclei because e.g. in some meiocytes fused tripolar anafase II spindles were found. Other deviating products may contain two haploid nuclei or no nucleus at all. No deviating large tetrads were found, implying that there had been no tetraploid PMC's.





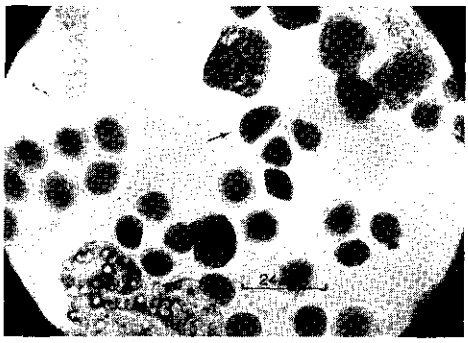


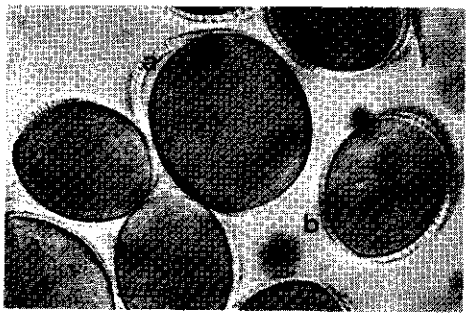
Fig. 1. Unreduced PMC (see arrow) and tetrads.
Fig. 2. Dyad
Fig. 3. Triad

Table 1. Distribution (%) of pollen grains over a scale for dimensions. m = mean; σ^2 = variance; ± 150 giant pollen grains and ± 300 normal pollen grains were measured. 1 unit = 3μm.

Dimension	Giant pollen grains		Normal pollen grains	
	length (m = 11.2, σ^2 = 0.38)	width (m = 10.6, σ^2 = 0.34)	length (m = 8.9, σ^2 = 0,09)	width (m = 8.5, σ^2 = 0.22)
7.0				1
7.5				2
8.0			6	34
8.5			9	24
9.0		2	79	39
9.5		1	6	
10.0	9	34		
10.5	8	18		
11.0	44	39		
11.5	22	3		
12.0	16	3		
12.5				
13.0				
13.5				
14.0	1			

Research on pollen grains. Examination of the pollen grains revealed that besides normal pollen grains, deviating pollen grains occurred which were much larger than the normal ones. In *Brassica* this was also found by FUKUSHIMA (1930), TOKUMASU (1965) and HEYN (1973). In other genera, such as *Amaranthus* (PAL & KHOSHOO, 1972) and *Arabis* (BÖCHER, 1951) they also occurred. In Table 1 the length and width of normal and deviating (giant) pollen grains are shown. From this table it appears that the dimensions of both types differed significantly. The giant pollen grains probably originated from the deviating products of sporogenesis mentioned above. They occurred in the different plants with unequal frequency.

Generative nuclei of the giant pollen grains, stained with chloral carmine, were also investigated. It appeared that a number of these pollen grains had two generative nuclei (see Fig. 4). The size of these nuclei was significantly greater than that of haploid nuclei in normal (trinucleate; BREWBAKER, 1967) pollen grains. This implies that these generative nuclei may be diploid and result from products of sporogenesis which were diploid owing to deviations in karyokinesis (first or second meiotic division) or absence of meiosis. Unreduced gametes were probably also formed on the female side because in my experiments the interdiploid cross *Brassica oleracea* × *Brassica rapa* yielded triploid hybrids (genomes ACC). Part of the giant pollen grains had four generative nuclei (see Fig. 5). The size of these nuclei was equal to that of haploid generative nuclei in normal pollen grains, suggesting that these nuclei were haploid. The pollen grains with four generative nuclei may originate from deviating products of sporogenesis (dyads or triads) arising from deviations in cytokinesis, whereas karyokinesis probably occurred normally. This implies that cytokinesis is independent of karyokinesis to some extent. For different plants the frequency in



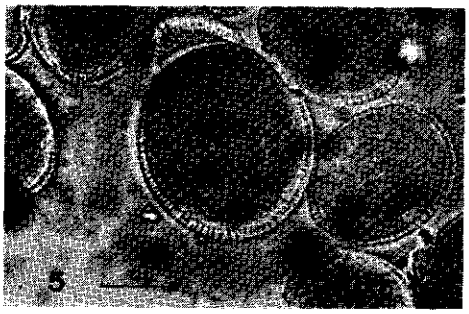


Fig. 4. Giant pollen grain (a) with 2 diploid generative nuclei and a normal pollen grain (b) with two haploid generative nuclei.
Fig. 5. Giant pollen grain with 4 haploid generative nuclei.

which the two types of giant pollen grains occurred was variable. Giant pollen grains with two generative nuclei were found more often (60%–100% of all giant pollen grains) than giant pollen grains with four generative nuclei. Giant pollen grains with two haploid generative nuclei or without generative nuclei were not observed.

Research on a correlation between the occurrence of giant pollen grains and matromorphic seeds. Table 2 shows the numbers of all seeds (a) and the numbers of matromorphic seeds (b) per 100 prickle pollinated buds of eleven clones and their frequencies of giant pollen grains (c). From this table it appears that great differences occur between clones for i.p.a. and frequency of giant pollen grains.

After arc sin transformation of the figures in Table 2 coefficients (r) have been computed for the correlation between a and c and between b and c. It was found that no significant correlation occurred between a and c ($r_{ac} = 0.26$) as contrasted with

Table 2. Seeds formed after prickle pollination and giant pollen grains of eleven clones of the variety Kolos.

Clone No.	Number of seeds per 100 prickle pollinated buds (not corrected for germination capacity)	Number of matromorphic seeds per 100 prickle pollinated buds	Number of giant pollen grains per 100 pollen grains
1	0.14	0	0.10
2	0.16	0.08	0.05
3	0.08	0	0.06
4	0.55	0.39	0.10
5	0.21	0.11	0
6	0	0	0
7	0.66	0.66	21.53
8	0.50	0.20	0.37
9	2.46	0.12	0.22
10	0	0	0.35
11	0.22	0	0.12

the correlation between b and c which was significant ($r_{bc} = 0.65^{+}$). RUTISHAUSER (1948) and CLAUSEN et al. (1961/1962) also found a positive correlation between the occurrence of apomixis and unreduced micro gametes in *Potentilla* and *Poa* respectively. The significant positive correlation found here is caused by the presence of clone 7; without the figures for this clone the correlation between b and c is low and not significant ($r_{bc} = 0.14$). This may be caused by various factors, such as: 1) differences between the clones for the relative frequency of deviations in cytokinesis and karyokinesis, 2) differences between macrosporogenesis and microsporogenesis for the relative frequency of different types of deviations (e.g. found in *Taraxacum*; GUSTAFSSON, 1935, 1938 and RUTISHAUSER, 1969) and 3) the obscuring effects of factors not involved in the origin and presence of unreduced gametes, but necessary for matromorphic development.

CONCLUSIONS

Absence of meiosis or deviations in cytokinesis or karyokinesis during meiosis resulted in the formation of unreduced PMC's, dyads and triads. These deviating products of sporogenesis probably correspond with giant pollen grains of which a high percentage had diploid (unreduced) generative nuclei. The observed relation between the occurrence of giant pollen grains and matromorphic seeds suggests similar deviations in macrosporogenesis of the investigated *Brassica oleracea* plants. Therefore diploid heterozygous matromorphic embryos may develop from deviating, diploid, products of gametogenesis. This conclusion agrees with results of earlier research (EENINK, 1974b, 1974c, 1974d).

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