

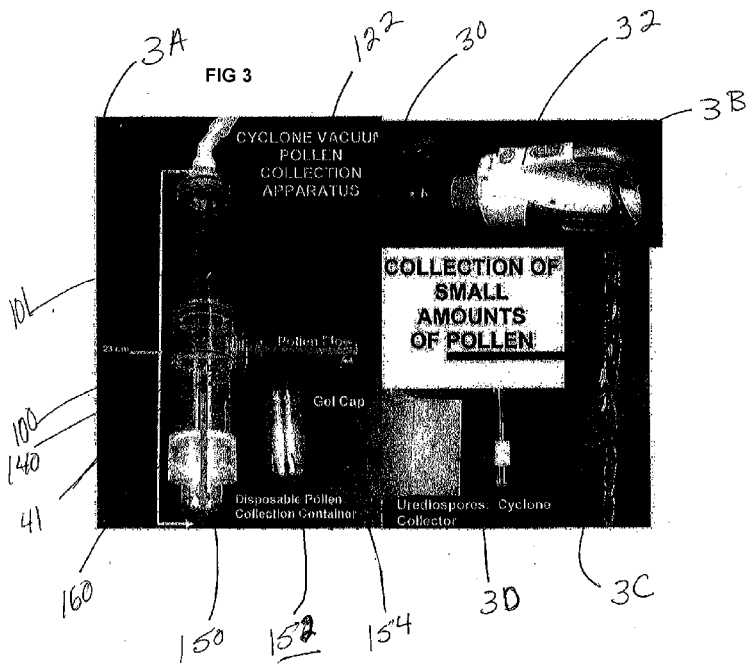


- (51) International Patent Classification:
A01H 1/02 (2006.01)
- (21) International Application Number:
PCT/US2012/028833
- (22) International Filing Date:
12 March 2012 (12.03.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/451,953 11 March 2011 (11.03.2011) US
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: VACUUM COLLECTION OF POLLEN AND ANthers FROM HAPLOID AND DOUBLED HAPLOID PLANTS



(57) Abstract: A method for collecting increased amounts of viable pollen from the anthers of a corn plant is disclosed. A cyclonic chamber having a vacuum port, a pollen collection port, and a pollen deposition port is connected to a source of a vacuum at the vacuum collection port. The vacuum created a vacuum at the pollen collection port which is passed adjacent the anthers of the plant to draw into the cyclonic chamber pollen from the anthers that would not be shed naturally by the anthers. The collected pollen is deposited in a reservoir connected to the deposition port of the cyclonic device where it is viable for the pollination of the same or another plant.

WO 2012/125593 A2

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

**VACUUM COLLECTION OF POLLEN AND ANTHERS FROM HAPLOID
AND DOUBLED HAPLOID PLANTS**

Reference to Related Applications

- [0001] This application claims the priority benefit under Title 35, United States Code 119(e) of United States Provisional Patent Application No. 61/451,953 filed March 11, 2011.

Background of the Invention

- [0002] The present invention relates generally to a plant breeding techniques and, more specifically, to a system for collecting pollen and anthers from plants, particularly from haploid and double haploid plants that typically produce small amounts of viable pollen.
- [0003] An understanding of the biological mechanisms which influence reproduction and development of plants, including flowering, provides a means for inducing haploids and generating doubled haploids. Species for which manipulation of reproductive systems and/or purity characteristics may be advantageous includes all crops, with important examples being the cereals, rice and maize, and wheat, barley, oats and rye in the temperate climates. Important crops for seed products are oil seed rape and canola, maize, sunflower, soyabean and sorghum. Many crops which are harvested for their roots are, of course, grown annually from seed and the production of seed of any kind is very dependent upon the ability of the plant to flower, to be pollinated and to set seed this seed. The timing of silking and pollen development, including the characteristics of anther extrusion and the amount and timing of production of viable pollen in flowering, is important. Horticultural plants whose flowering and particularly their pollen production may be manipulated for plant development and breeding purposes include lettuce, endive and vegetable brassicas including cabbage, broccoli and cauliflower, and carnations and geraniums. Double haploid pollen production limits or enables plant development and breeding processes which are that often characterized as successful or not based on the amount of seed that is set on the plants. Seed set is greatly influenced by the amount of viable pollen from the haploid or partial doubled haploid or the doubled haploid that actually reach the silks and ultimately the egg.
- [0004] In many instances, tassels and other pollen producing structures on haploid and chimeric (diploid/haploid) doubled haploid corn, wheat, barley, rice, etc. and other

haploid plants produce very small amounts of viable pollen. In this regard, the conventional method of pollen collection, which involves putting a bag over the tassel, is usually an inadequate method for pollen collection, particularly when small amounts of viable pollen have been produced, as is often the case in haploid and doubled or partially doubled haploid and some doubled haploid plants. The present invention provides a means to collect viable pollen from fertile anthers of haploid and doubled haploid, or other corn plants producing small amounts of viable pollen, and use the collected pollen for direct placement onto the receptive stigmas (silk) to produce seed.

Summary of the Invention

- [0005] The present invention represents a novel apparatus and method to collect small amounts of viable corn pollen from tassels of corn plants, particularly pollen producing anthers on plant structures which may include the tassels of haploid and doubled haploid plants.
- [0006] The method of double haploid breeding in particular maize or corn plants comprises the steps of: growing a corn plant and cross pollinating the plant with a haploid inducing plant, harvesting haploid seed. Growing said seed to form plant material and exposing said plant material to a doubling agent and growing said doubled or partially doubled haploid plants to a stage of pollen viability in the anthers and providing a cyclonic chamber having a vacuum port, a pollen collection port, and a pollen deposition port; providing a source of a vacuum connected to the vacuum collection port; passing the pollen collection port adjacent the anthers of the plant to draw into the cyclonic chamber pollen from the anthers; depositing the collected pollen in a reservoir connected to the deposition port of the cyclonic device; and pollinating the same or another corn plant with the collected pollen wherein the seed of said plant is viable seed, and growing said seed to form a plant for further selfing or further breeding and or development.
- [0007] More specifically, the present invention includes a device consisting of (a) a nozzle to direct the collection of pollen, (b) a vacuum source, (c) a cylinder capable of generating a cyclonic air movement, and (d) a disposable receptacle in which to collect viable corn pollen. In this pollen collection system, the pollen is captured via cyclonic air movement wherein the viable pollen grains settle out of the air stream and are collected in a receptacle. The viable corn pollen is then placed directly onto the receptive stigmatic tissues (silks). The present inventions uses the

method for collecting pollen from anthers of a plant, comprising the steps of providing a cyclonic chamber having a vacuum port, a pollen collection port, and a pollen deposition port, providing a source of a vacuum connected to the vacuum collection port, passing the pollen collection port adjacent the anthers of the plant to draw into the cyclonic chamber pollen from the anthers that would not be shed naturally by the anthers; and depositing the collected pollen in a reservoir connected to the deposition port of the cyclonic device where it is viable for the pollination of the same or another plant.

[0008] Another method of the present invention is useful for breeding monocot plants comprising barley, rye, triticale, wheat, rice and maize. The method of breeding in particular maize or corn plants comprises the steps of: growing a corn plant to a stage of pollen viability in the anthers; providing a cyclonic chamber having a vacuum port, a pollen collection port, and a pollen deposition port; providing a source of a vacuum connected to the vacuum collection port; passing the pollen collection port adjacent the anthers of the plant to draw into the cyclonic chamber pollen from the anthers; depositing the collected pollen in a reservoir connected to the deposition port of the cyclonic device; and pollinating the same or another corn plant with the collected pollen.

[0009] A novel feature of this apparatus is that it provides a means to collect very small amounts of viable pollen generated on haploid and doubled haploids plants, each of which may generate a limited number of functional anthers. In addition, the collected viable corn pollen grains can be placed directly onto the silks.

Brief Description of the Figures

[00010] Fig. 1 is photograph of an embodiment of the present invention.

[00011] Fig. 2 is a photograph of an alternative embodiment of the present invention wherein a hand graspable pistol grip is modified to provide the features of the present invention.

[00012] Fig. 3 is a composite photograph showing a vacuum pump for use with the present invention, a cyclone vacuum pollen collection apparatus, collected urediospores, and a photomicrograph of an anther which is producing only a small amount of pollen.

[00013] Fig. 4 is a photograph of two ears that were produced using pollen collected by the present invention.

[00014] Fig. 5 is a photograph of a device of the present invention showing pollen collected from a pictured diploid corn plant wherein the anthers did not dehisce well.

Detailed Description of a Preferred Embodiment

[00015] Due to their chimeric nature (mixture of haploid and diploid cells) many doubled haploid corn plants produce inadequate amounts of viable pollen. Some haploid and doubled plants may have spontaneous sectors of the tassel bearing fertile flowers, but often these regions are infrequent and may produce limited amounts of viable pollen. As such, self pollination and seed production is often not possible with the above described haploid and doubled haploid plants.

[00016] Even when adequate amounts of pollen are produced on a haploid or doubled haploid plant, the conventional method of pollen collection in maize for example is to use a tassel bag. A tassel bag may be problematic due to any or all of (a) the plant not being large enough to support the weight of the bag, (b) the plant not being large enough to tolerate the increased wind-based movement of the plant due to the sail-like effect of the bag, or (c) the inability of the operator to remove a suitable amount of pollen from the bag. In addition, removal of limited amounts of viable pollen from the bag onto the receptive silk tissue can be problematic in a field environment due to inadvertent release of pollen from the pollen bag due to having to manipulate a large tassel bag amongst the leaves of adjacent plants. In addition, unless the tassel bag is immediately adjacent to the ear silks, wind movement can blow the pollen away from the silks. In each of these examples, reduced seed production can result.

[00017] The present invention provides a means to easily collect and distribute significant amounts viable pollen from haploid or doubled haploid plants directly onto the ear silks and, thus, results in more haploid and doubled haploid plants producing homozygous seed and the production of significantly greater amounts of seed per ear.

[00018] Some of the problems in doubled haploid corn are that some tassels on doubled haploid corn plants produce only a few fertile florets, there may be a larger number of only partially fertile florets, and the extruded anthers do not dehisce well. In any of these cases, making an effective self pollination with standard hand pollination techniques is problematic because it is difficult to collect and distribute a limited number of viable pollen grains onto receptive silk tissues.

A cyclonic rust spore collector was purchased from G-R manufacturing, 1317 Collins Lane, Manhattan, Kansas 66502-9577 for research purposes. In cereals and oats in particular, the study of rust disease has led to the development of a urediospore collector. This device operates via a cyclone vacuum system to collect rust spores in gel capsules (Fig. 1). In essence, the system is used to vacuum-up urediospores from the surfaces of leaves. Figure 1 is a drawing of an embodiment of the Cyclonic rust spore collector which is used in the method of the present invention which shows a nozzle 20 which is used not for spores but to direct the collection of pollen 22. In this embodiment there is shown a vacuum line 30 which is connected to the cyclonic cylinder 40 vacuum source 32 (shown in Figure 3) which is not shown in Figure 1. The nozzle 20 is connected to a cyclonic cylinder 40 which is capable of generating a cyclonic air movement. This cyclonic air movement acts to deposit the pollen 22 in the disposable receptacle 50. In figure 1, the disposable receptacle is a half of a pill or a single gel cap 52 similar to the gel capsules 54 used for providing medicine to large farm animals. In the present method of pollen collection in this system 10, the pollen 22 is captured via cyclonic air movement 42 (shown in Fig 3) wherein the viable pollen grains 22 settle out of the air stream and are collected in a receptacle 50. The use of a disposable receptacle 50 significantly decreases any issues of cross contamination of pollen between pollen collections. Additionally, the vacuum system is cleansed of extraneous pollen by running the vacuum system for short periods without a receptacle attached between collecting pollen from different plant genotypes. The viable corn pollen is then placed directly onto the receptive stigmatic tissues (silks) (not shown).

Figure 2 shows a alternative embodiment of the present invention, which is based on the ease of use of a automated pipetting device 300. The figure is an automated battery operated pipetting system that releases set amounts of material. The design of the hand shaped gun system would work well for pollen collection in both green houses but also in fields. The vacuum power could be turned off and on by with the grip of the hand, leaving the other hand free to collect and replace receptacles from the device. Alternatively, the receptacle replacement could be automated in a cartridge system where each receptacle has a second gel cap pushed onto it to form an enclosed capsule once the receptacle is filled. The enclosed gel capsule is shown in Figure 3a.

Figure 3 shows four different figures 3a, 3b, 3c, and 3d. Figure 3b shows a vacuum pump 32 for use in the method of the present invention. This vacuum source can be transported or carried in a back pack, strapped to a forearm, slung around the shoulder or neck with an attached strap. The vacuum line 30 in Figure 3B attaches to the improved cyclone column 101 with the cyclonic cylinder 140 below. The vacuum pollen collection apparatus shown in Figure 3a has the vacuum line 30 attached on one end to the vacuum source 32 and the other end is attached to the improved cyclone column 101 with the nozzle 122 located approximate the cyclonic cylinder 140. The cyclonic cylinder 140 optionally has dual detachable collectors 150 and 160.

This improved vacuum system 100 allows for less clogging and decreases any associated damage to the pollen from the prior art device of figure 1. The device 100 is approximately 23 cm in size. The size can be altered but it must be capable of free pollen movement and reduced pollen damage. In comparison to the Figure one device the present invention has a larger area for pollen flow in the nozzle 122 and cyclonic air movement shown by two arrows 41 in Fig 3a, with dual detachable collectors 150 and 160 (160 shown in Figure 5) to decrease pollen contamination. The

dual detachable collectors fulfill two important goals. The first goal is to allow for larger amounts of pollen collection than can be achieved by the disposal collector 150, and the second goal is to allow for additional cleaning of the pollen collection area by removing the larger collector portion 160. Figure 3D in comparison shows that the urediospores cyclone collector is not well adapted for larger amounts of pollen which may need to be collected. Figure 3c shows the relative size of the tassel and the anthers that are on a maize tassel, similar anthers are on wheat, oat, barley, triticale and rye spikes and rice panicles.

Figure 4 shows a photograph of the two ears that were produced from pollen collected using the method of this invention and the device of Figure 3a. This device collected substantially undamaged viable pollen. The adapted device permits anthers to be collected even from plant anthers that did not dehisce well.

The present inventions uses the method for collecting pollen from anthers of a plant, comprising the steps of providing a cyclonic chamber having a vacuum port, a pollen collection port, and a pollen deposition port, providing a source of a vacuum connected to the vacuum collection port, passing the pollen collection port adjacent the anthers of the plant to draw into the cyclonic chamber pollen from the anthers that would not be shed naturally by the anthers; and depositing the collected pollen in a reservoir connected to the deposition port of the cyclonic device where it is viable for the pollination of the same or another plant.

[00019] Because both corn pollen and rust urediospores represent very small biological structures, it was conceived that perhaps a urediospore-like collector might be used to collect and make pollinations from doubled haploid tassels where very little viable pollen is produced.

EXAMPLE 1

[00020] This experiment sought to determine if pollen could be collected via a rust spore collector and whether or not the pollen captured in this manner remained viable and capable of functioning to produce seed.

Materials and Methods

[00021] Pollen was collected in a tassel bag from a shedding diploid tassel in the greenhouse and brought into the lab. The pollen was placed on the surface of a piece of glassine weigh paper. A rust spore collection device was hooked to the lab vacuum system and the input tube of the cyclone collector was placed near the pollen. A small amount of the pollen was sucked into the vacuum system and via cyclonic air movement, deposited in the gel cap. The pollen was allowed to move in a circular motion within the gel cap for approx. 30 seconds. The gel cap containing the pollen was then transferred directly back to the greenhouse and used to pollinate an ear.

Results

[00022] Over 50 seed were produced on the ear. Seed production on the ear demonstrates that it is indeed possible to collect corn pollen in a cyclone vacuum system in such a way such that pollen viability is retained and collected pollen can be used to produce seed.

Conclusion

[00023] These results support using a vacuum system to aid in the collection of small amounts of pollen that become available on the tassels of haploid and partially doubled and doubled haploid corn plants. Vacuum collection of pollen significantly improves seed production on doubled haploid plants because current methods of self pollination are not particularly well suited for situations where limited amounts of viable pollen are available. As noted above, some partially fertile anthers on doubled haploid tassels do not dehisce well, and thus, self pollination becomes problematic due to the lack of pollen shed. A cyclonic system in which the anthers spin around in the collection receptacle for a period of time provides an opportunity to break open the anthers in such a way that the viable pollen grains may exit the anther and subsequently be collected (along with other pollen grains) in the pollen collecting receptacle. In this way, seed production should be possible on haploid, partially doubled haploids and doubled haploid plants that typically would be problematic if not impossible to self-pollinate.

EXAMPLE 2

[00024] Doubled haploid maize plants may extrude limited amounts of functional/fertile anthers. Typically, pollen from doubled haploid plants is collected from the tassel in a bag. To produce doubled haploid seed, the pollen then is distributed over the top of brush of silks on that same plant. However, when limited numbers of fertile anthers are present on a tassel, the process of collecting pollen in a bag and distributing that pollen on a brush of silks can be problematic. The purpose of this experiment was to evaluate the cyclonic pollen collection device (Fig. 3) in terms of its ability to collect pollen from corn tassels, and test how viable and functional the pollen is post collection by using the assembled pollen to pollinate ears of corn, as measured by seed production.

Materials and Methods

[00025] The pollen collection device consists of a vacuum source, a cyclonic tube shaped container to separate the pollen from the vacuum airstream, a nozzle used to direct a vacuum to regions of the tassel in order to remove the pollen and anthers from the tassel, and a disposable gel cap which is used to collect the pollen.

[00026] Pollen was collected from a fresh haploid inducer tassel by turning on the vacuum source and moving the nozzle of the pollen collection device along several of tassel branches of a haploid inducer tassel. Pollen was collected in this fashion several times in order to collect enough pollen to pollinate several ears. The G7 gel cap, now containing the collected pollen, from the pollen collection device was removed and the collected pollen was used to pollinate four. For the first and third pollination the pollen was sprinkled out of the gel cap and onto ears with a large amount of silks. For the second and fourth pollination the gel caps were turned upside down, placed over top of the silks, which brush the inside of the gel cap and the pollen was rubbed on the silks. Five ears were also pollinated the traditional way with the pollen collected from a pollen bag.

[00027] Fifteen days after pollination the nine ears were harvested and the kernels from each ear were counted.

Results

[00028] The results are presented in Table 1.

Table 1

Number of kernels from pollinating in the traditional way	Number of kernels from pollen collected from the pollen collector and sprinkled over the ears	Number of kernels from pollen collected from the pollen collector and the gel cap turned upside down and rubbed on the silks
34	0	78
210	33	95
18	x	x
65	x	x
179	x	x

[00029] The pollen collection device worked quite well to collect and deposit pollen in the gel cap container. The device was light weight and easily handled amongst the plants. Pollen and anthers were easily sucked into the nozzle of the device as it was moved up and down along the length of each tassel branch. The vacuum air stream from the nozzle as well as rubbing the nozzle against the

tassel branch helped to dislodge anthers and shake loose pollen, both of which resulted in very good pollen collection

[00030] Large amounts of pollen and anthers were quickly collected in this fashion. A static charge seemed to adhere some of the pollen to the gel cap wall, but the pollen was easily removed by inserting the silk brush into the gel cap and twisting the gel cap. A large amount of collected pollen within the gel cap made it possible to also pour out the pollen onto ear silks. Seed production was variable from ear to ear, but was not atypical with respect to our observation of seed production from conventional hand pollination using a tassel bag.

EXAMPLE 3

[00031] Fig. 2 is a picture of the hand-operated vacuum collector. In this case, a cyclonic action is incorporated into the device in order to collect pollen, possibly into a disposable collection receptacle. The device is ergonomically modeled to look something like existing hand-held auto-pipetters. This device could be used to both collect and transfer pollen from the tassel to the silk. The belt or backpack vacuum pump and energy source would be connected to this hand-held pollen collection unit via tubing.

[00032] The pollen collection device readily collected pollen and the collected pollen was viable and able to produce seed on pollinated ears at a level reasonably comparable to hand pollination.

[00033] The foregoing description and drawings comprise illustrative embodiments of the present inventions. The foregoing embodiments and the methods described herein may vary based on the ability, experience, and preference of those skilled in the art. Merely listing the steps of the method in a certain order does not constitute any limitation on the order of the steps of the method. The foregoing description and drawings merely explain and illustrate the invention, and the invention is not limited thereto, except insofar as the claims are so limited. Those skilled in the art that have the disclosure before them will be able to make modifications and variations therein without departing from the scope of the invention.

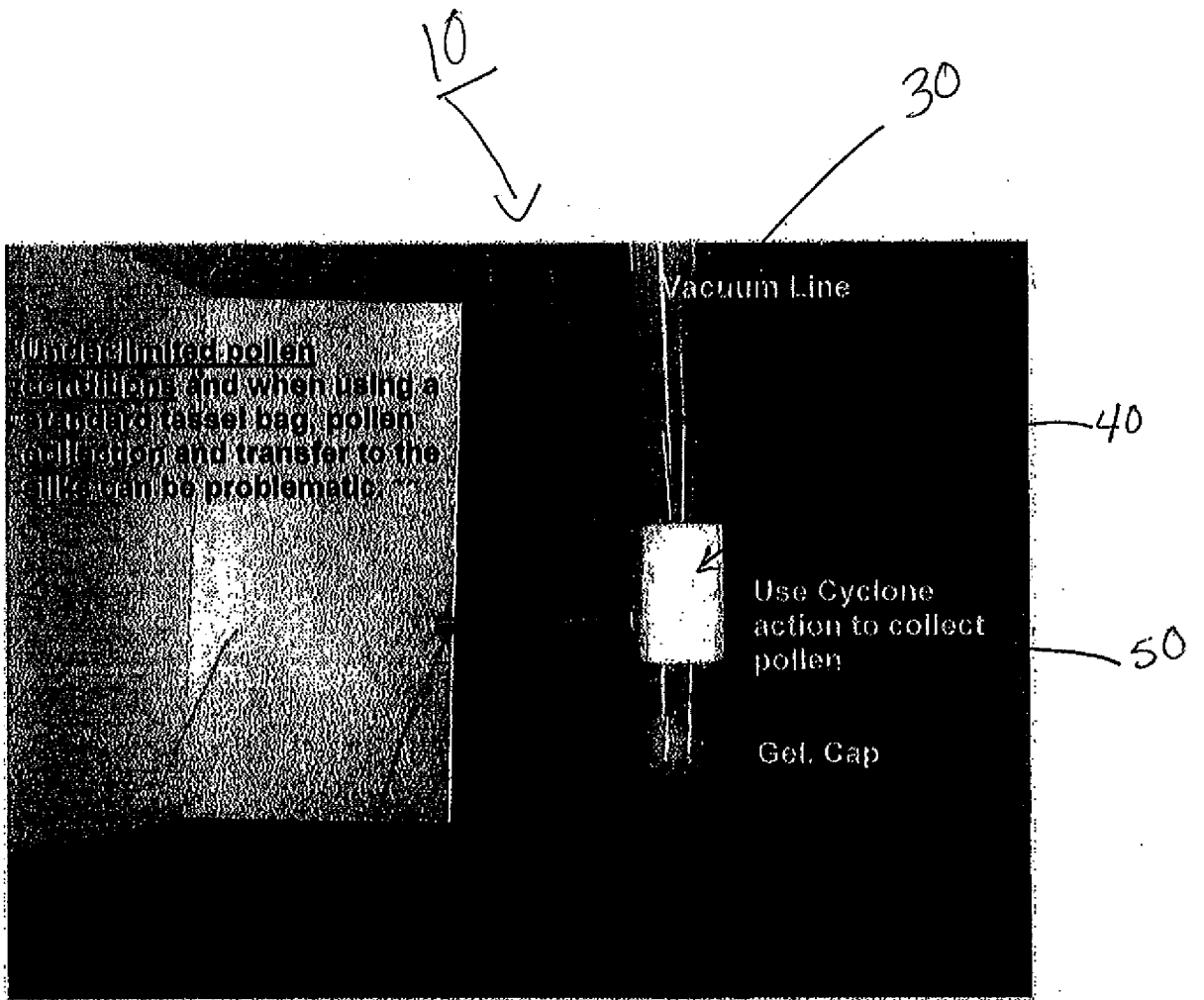
We claim:

1. A pollen collection apparatus to collect small amounts of viable pollen comprising a vacuum source, a cylinder capable of generating a cyclonic air movement, and a disposable receptacle in which to collect viable pollen from anthers of plants, particularly pollen producing anthers on plant structures of haploid, partially doubled haploid or doubled haploid plants.
2. A pollen collection device according to claim 1 where in the pollen is from anthers of a monocot.
3. A pollen collection device according to claim 1 where in the pollen is from anthers of a maize, wheat, barley, or rice which is a partially doubled haploid plant.
4. A pollen collection apparatus to collect small amounts of viable maize pollen comprising a vacuum source, a cylinder capable of generating a cyclonic air movement, and a disposable receptacle in which to collect viable pollen from anthers of plants, particularly pollen producing anthers on plant structures of haploid, partially doubled haploid and doubled haploid plants.
5. A pollen collection apparatus according to claim one further comprising (a) a nozzle to direct the collection of pollen.
6. A pollen collection apparatus according to claim 1 wherein the pollen is captured via cyclonic air movement of said cylinder.
7. A pollen collection apparatus according to claim 6 wherein the pollen captured via cyclonic air movement comprises at least some viable pollen grains.
8. A pollen collection apparatus according to claim 7 wherein some of said viable pollen grains settle out of the air stream and are collected in a receptacle.
9. A pollen collection apparatus according to claim 6 further comprising a pollen receptacle.
10. The viable pollen collection apparatus according to claim 8 wherein the pollen in said receptacle has an open end which is placed proximate to or surrounds the silks when distributing the pollen onto the receptive stigmatic tissues (silks).

11. A method for collecting pollen from anthers of a plant, comprising the steps of:
 - (a) providing a cyclonic chamber having a vacuum port, a pollen collection port, and a pollen deposition port;
 - (b) providing a source of a vacuum connected to the vacuum collection port;
 - (c) passing the pollen collection port adjacent the anthers of the plant to draw into the cyclonic chamber pollen from the anthers that would not be shed naturally by the anthers; and
 - (d) depositing the collected pollen in a reservoir connected to the deposition port of the cyclonic device where it is viable for the pollination of the same or another plant.
12. A method for breeding corn plants, comprising the steps of:
 - (a) growing a corn plant to a stage of pollen viability in the anthers;
 - (b) providing a cyclonic chamber having a vacuum port, a pollen collection port, and a pollen deposition port;
 - (c) providing a source of a vacuum connected to the vacuum collection port;
 - (d) passing the pollen collection port adjacent the anthers of the plant to draw into the cyclonic chamber pollen from the anthers; and
 - (d) depositing the collected pollen in a reservoir connected to the deposition port of the cyclonic device; and
 - (e) pollinating the same or another corn plant with the collected pollen.
13. The method for breeding corn plants according to claim 12 wherein the corn plant is a double haploid plant said method further comprising the steps of developing said double haploid corn plant by growing a corn plant and cross pollinating the plant with a haploid inducing plant and harvesting haploid seed.
14. The method for breeding corn plants according to claim 13 further comprising the step of growing said haploid seed to form plant material; exposing said plant material to a doubling agent; and growing said doubled or partially doubled haploid plants to a stage of pollen viability in the anthers.
15. The method for breeding corn plants according to claim 14 wherein the pollinated corn plant produces viable seed, and further comprising the step of

growing said viable seed to form a plant for selfing or breeding and or development.

16. The seed of the method of claim 15.



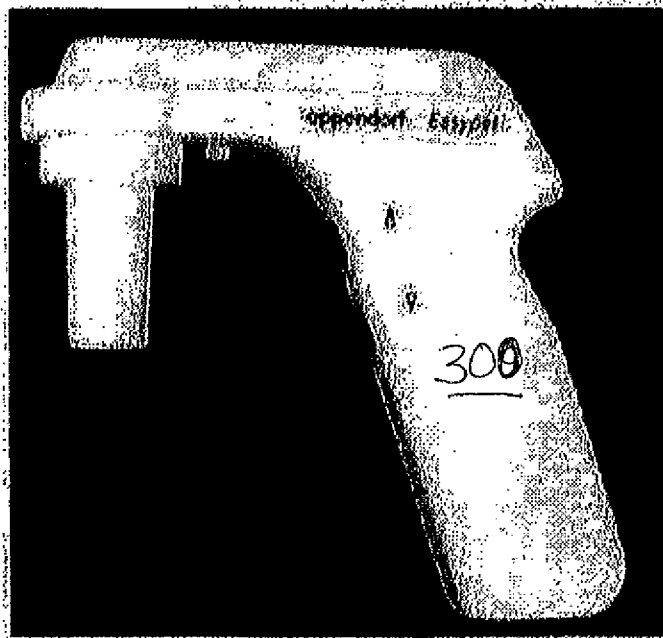


FIG. 2

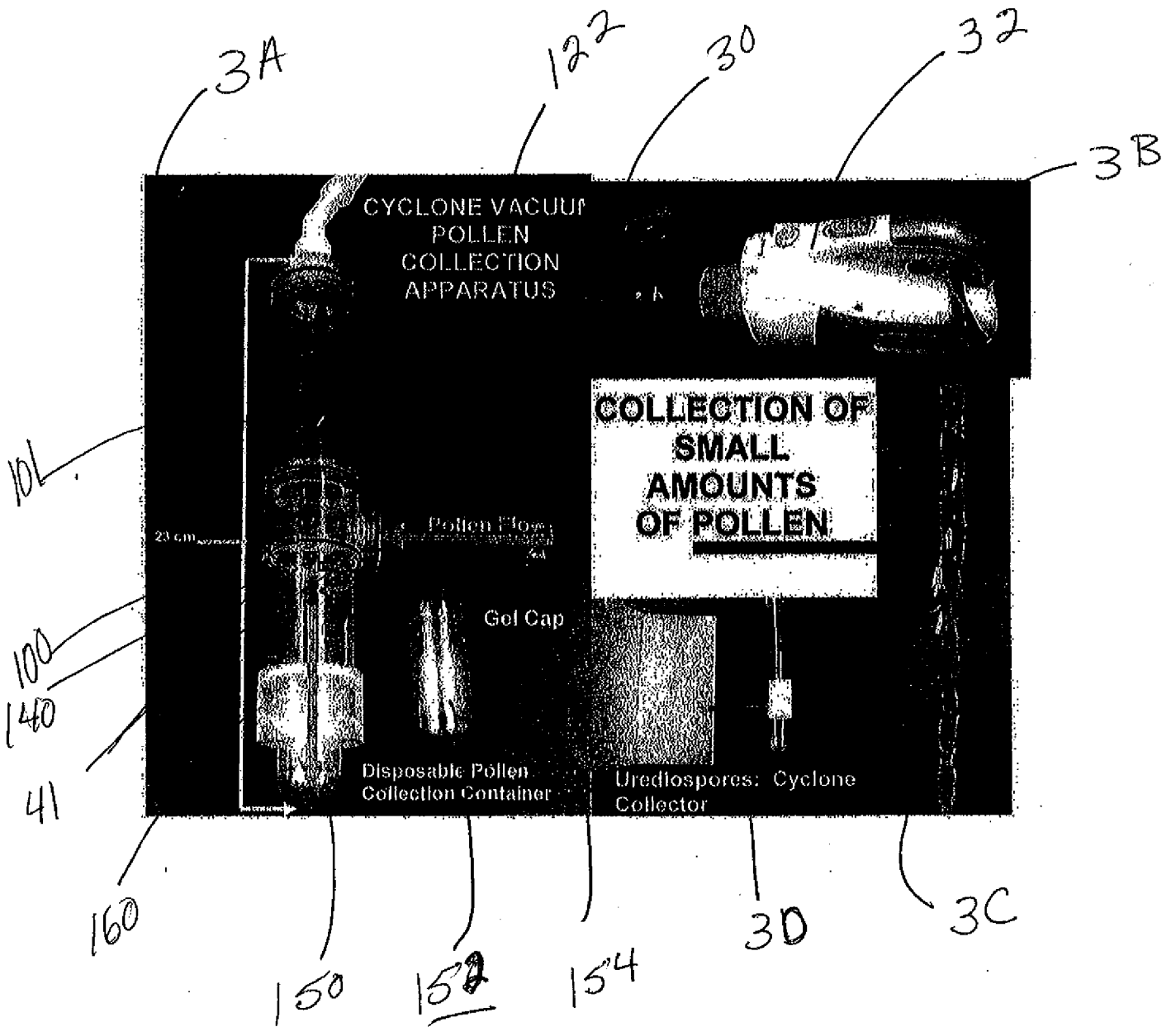


FIG 3

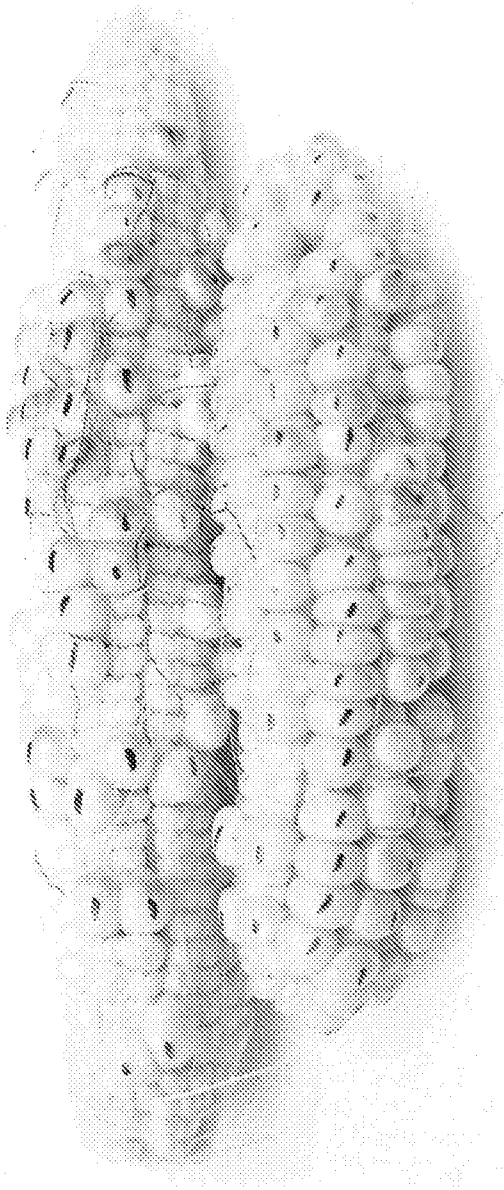


FIG. 4

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FIG. 5