FERTILISATION INDEPENDENT FRUIT FORMATION IN EGGPLANT

The present invention relates to an eggplant seed that is capable of growing into a plant that is cytoplasmic male sterile and can produce eggplant fruits without fertilization. The present invention also relates to a plant produced by growing the eggplant. The invention further relates to methods for producing the seedless eggplant fruits.

Figure 1: A. Flowers with normally opening anthers of non-CMS plants. B. Flowers with deformed non-opening anthers of CMS plants.

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FERTILISATION INDEPENDENT FRUIT FORMATION IN EGGPLANT

The present invention relates to a seedless eggplant and to a method for producing such a plant.

The eggplant or aubergine (Solanum melongena) is a member of the Solanaceae plant family and part of the Solanum genus which comprises more commercially interesting species such as tomato, potato and pepper. Because of its large, pendulous, purple or white fruit, the eggplant is an important food crop. It has been cultivated in southern and eastern Asian countries since prehistory but is now also grown commonly in the Western world.

The fruit contains numerous small, soft seeds. From a commercial point of view seedlessness is a very desirable trait in edible fruit and vegetables, like the eggplant. The presence of seeds in ready-to-harvest fruits is considered as a negative quality. This is inter alia because seeds can lead to undesired browning of the flesh. Furthermore, seedless fruits are in general more sweet than fruits with seeds. Industrial or domestic applications which require seed removal from the fruits can also benefit strongly from the absence of seeds.

Seedlessness or the absence of seeds can be the result of parthenocarpic fruit formation, where fruits set without fertilization. In certain species parthenocarpic fruit set still requires pollination or another stimulus, in particular spraying the flowers with plant growth regulators, such as gibberellin, auxin and cytokinin. This is termed artificial parthenocarpic fruit formation.

Parthenocarpic fruit formation is not only interesting for obtaining seedless fruits. Unfavourable environmental conditions such as high or low temperatures and drought can hamper normal pollination which leads to poor fruit set and as a consequence yield loss. When fruit formation independent of fertilization or parthenocarpic fruit formation can be harnessed as a trait, it could significantly contribute to an economically more efficient production of eggplant fruits.

In addition to contributing to harvest security, parthenocarpic fruit formation is also important for fruit quality. Parthenocarpic eggplant fruits have been mentioned to have a better flavour (less bitter) as well as a higher dry matter content as compared to seeded eggplant fruits. The higher level of soluble solids is especially important for processing eggplants which are used in industry for paste production. In addition, such fruit can be advantageous for the fresh cut industry which requires firm fruits which are not leaky.

Several parthenocarpic eggplant varieties are known (e.g. Talina, Galine). During winter cultivation of such eggplant varieties fruit production may be hampered by suboptimal environmental conditions. These are usually counteracted by treating flower buds with plant growth regulators. However, these phytohormonal treatments make the production process more expensive due to the cost of both chemicals and labour. The involvement of the plant hormones auxin and gibberellin has been extensively documented although their precise role remains elusive.
The application of either auxin or gibberellin to the unfertilised ovule leads in many plant species including eggplant to fruit formation. In practice, these hormones are applied to improve fruit set when greenhouse conditions are suboptimal. Although the application of auxin and gibberellin has some practical value it increases costs and it may lead to irregularities in fruit shape. In addition, the use of hormones is under discussion or prohibited in some countries.

Parthenocarpic fruit formation has also been genetically engineered in eggplant by using the DefH9-iaaM gene. The DefH9-iaaM gene codes for tryptophan monoxygenase and confers auxin synthesis, while the DefH9 controlling regions drive expression of the gene specifically in the ovules and placenta. This leads to a significant increase in fruit production concomitant with a reduction in cultivation costs. However, in many countries genetically modified crops are not well accepted.

Besides the above described drawbacks of the current parthenocarpic eggplants and methods available, there is another issue. An eggplant that possesses the trait parthenocarpic fruit formation can still produce fruits that contain seeds if the eggplant was able to self-pollinate in certain conditions. The quality of the eggplant fruits can be negatively influenced by the presence of seeds. As a consequence, in case one desires guaranteed seedless pepper fruit, manual emasculation (removal of anthers before maturation) of the pepper plant flowers is a necessity even if the plant itself is parthenocarpic.

The prevention of self-pollination is also an issue in the production of F1 hybrid seeds. The flowers of eggplants are complete, containing both female and male structures, and may be self-pollinated or cross-pollinated. This self-pollination is non-desirable in F1 hybrid seed production, because F1 hybrid seed is produced after crossing of two different plant lines. The unwanted self-pollination can be controlled by laborious manual emasculation. This emasculation is done to prevent a plant from producing pollen so that it serves only as a female parent. Emasculation is time- and cost consuming and thus undesirable.

Another approach for producing seedless eggplant fruits is the use of triploid eggplants. Triploid eggplant exist that can produce seedless eggplant fruits (see WO2009/095266). A triploid eggplant is produced by first crossing a tetraploid eggplant parent plant with another diploid eggplant and then selecting from the population of progeny plants the triploid plants. Preferably, the tetraploid parent is the mother plant and the diploid parent is the father plant. To obtain tetraploid parent plants, common diploid eggplants are given a colchicine treatment. The process of producing a triploid eggplant is laborious and bound by certain treatments.

It is the object of the present invention to provide an eggplant that can produce fertilization independent seedless fruits and that does not have any of the above stated drawbacks.

The current invention relates to an eggplant (Solanum melongena L.) that shows a combination of both parthenocarpic fruit formation and cytoplasmic male sterility (CMS).
The plant of the invention is a parthenocarpic plant that is unable to produce viable pollen. Since this male-sterile plant can thus not self-pollinate, seed formation is dependent upon pollen from another plant acting as a male plant in the cross. This way the occurrence of selfed progeny in F1 hybrid seed production is avoided. The use of a female sterile plant would not have the same benefits as male sterile plants in F1 hybrid seed production as emasculation would still be necessary.

The invention relates in particular to eggplant seed capable of growing into a plant that is cytoplasmic male sterile and capable of producing eggplant fruits without fertilization, which seed is obtainable by crossing a mother plant of NCIMB 42053 with a father plant that is optionally parthenocarpic.

Said eggplant seed can also be obtained by crossing a mother plant of NCIMB 42053 with a father plant, that is optionally parthenocarpic, and after this first cross repeatedly backcrossing the progeny plants with the recurrent father plant. By repeatedly backcrossing progeny plants with the recurrent father plant, it is possible to obtain eggplant plants that have the desirable traits or combinations of traits similar to the father plant plus the combined traits of cytoplasmic male sterility and parthenocarpic fruit formation. The invention also relates to the plants that can be grown from these seeds.

According to the invention different combinations of female and male parents could lead to a plant of the invention. However, because cytoplasmic male sterility (CMS) is transferred via the cytoplasm, this trait obviously can only be inherited via a plant acting as a female. To obtain a plant according to the current invention, the plant used as a female in the cross for obtaining the plant with the combination of CMS and parthenocarpic fruit formation in the crossing must always have the trait CMS. The parthenocarpic fruit formation is a trait that can be inherited both from male and female parent plants. To obtain a plant according to the invention as described above, one or both parent plants must have the trait parthenocarpic fruit formation.

The plant that functions as a mother plant in the cross that leads to seed of the invention, can be a plant which representative seed was deposited with the NCIMB under accession number NCIMB 42053. It could also be a plant derived from the plant of which representative seed was deposited under accession number NCIMB 42053 or a plant that has at least the same cytoplasmic male sterility as comprised in a plant of which representative seed was deposited with the NCIMB under accession number NCIMB 42053.

The invention also relates to an eggplant grown from said seed, which plant is cytoplasmic male sterile and capable of producing eggplant fruits without fertilization. Furthermore, said eggplant, which is male sterile and capable of producing eggplant fruits without fertilization, grown from said seed does not show any negative pleiotropic effects.

Moreover, the invention provides a seedless eggplant fruit produced from an eggplant that
is cytoplasmic male sterile and capable of producing fruits without fertilization.

This seedless eggplant fruit produced by a male sterile eggplant capable of producing fruits without fertilization, does not show any negative pleiotropic effects, in particular a hollow core and/or a deformed shape.

Furthermore, said seedless eggplant fruit has one or more of the following characteristics:

a) a size that is the same or similar to the size of a fruit produced through fertilization; b) a coloration and ripening process that is the same or similar to the coloration and ripening process of a fruit produced through fertilization.

The said seedless eggplant fruit can be the fruit itself, or a product or food product made of the fruit or made of parts thereof, or a processed food product made thereof, and is harvested from an eggplant of the invention or an eggplant grown from seed of the invention comprising the combination of parthenocarpic fruit formation and cytoplasmic male sterility.

The invention also relates to progeny of an eggplant as described above that has retained the cytoplasmic male-sterility and the capability of fertilization independent fruit formation of the parent. The cytoplasmic male sterility in said progeny of a plant of the invention is as found in seeds of which a representative sample was deposited under accession number NCIMB 42053. This applies both to the phenotype and the genotype, i.e. the genes underlying the CMS.

The invention further relates to propagation material derived from a plant of the invention or a plant grown from seed of the invention. An eggplant grown from said propagation material, has retained the cytoplasmic male-sterility and the capability of fertilization independent fruit formation. The trait cytoplasmic male sterility in said propagation material derived from a plant of the invention or a plant grown from seed of the invention is as found in seeds of which a representative sample was deposited under accession number NCIMB 42053.

The invention also describes propagation material capable of growing into a plant of the invention or a plant grown from seed of the invention. The propagation material derived from a plant of the invention or capable of growing into a plant of the invention can be selected from a group consisting of: microspores, pollen, ovaries, ovules, embryos, embryo sacs, egg cells, cuttings, roots, stems, cells, prooplasts, leaves, cotyledons, hypocotyls, meristematic cells, roots, root tips, microspores, anthers, flowers, seeds, callus, stems, tissue culture or parts thereof.

In one aspect the invention relates to a method for the production of an eggplant that shows a combination of the traits parthenocarpic fruit formation and cytoplasmic male sterility, comprising:

a) crossing a female parent plant comprising the trait of cytoplasmic male sterility with another recurrent male parent plant, wherein at least one of the parent plants has the trait of parthenocarpic fruit formation;
b) crossing the resulting F1 with the recurrent parent plant from step a) for obtaining F2 plants;

c) selecting plants that have the combination of cytoplasmic male sterility and parthenocarpic fruit formation in the F2;

d) optionally performing one or more additional rounds of crossing, and subsequently selecting, for a plant comprising/showing cytoplasmic male sterility and parthenocarpic fruit formation.

The words "trait" and "traits" in the context of this application refer to the phenotype of the plant. In particular, the word "traits" refers to the combination of both parthenocarpic fruit formation and CMS. When a plant shows the trait of the invention, its genome comprises genetic information causing the trait of the invention.

It is clear that the parent plant that provides the trait of the invention is not necessarily a plant grown directly from the deposited seeds. The parent plant can also be a progeny plant from the seed or a progeny plant from seeds that are identified to have the combination of traits of the invention by other means.

The invention additionally provides a method of introducing other desired traits into an eggplant which shows the combination of the traits parthenocarpic fruit formation and CMS, comprising:

a) crossing a first female eggplant that shows a combination of the traits parthenocarpic fruit formation and cytoplasmic male sterility, representative seed of which were deposited under deposit number NCIMB 42053, with a second eggplant that comprises a desired trait to produce F1 progeny;

b) selecting an F1 progeny that comprises a combination of said traits plus the desired trait;

c) crossing the selected F1 progeny with the second parent, to produce backcross progeny;

d) selecting backcross progeny comprising the desired trait plus the combination of parthenocarpic fruit formation and cytoplasmic male sterility; and

e) optionally repeating steps c) and d) one or more times in succession to produce selected fourth or higher backcross progeny that comprises the desired trait and the combination of said traits. The invention includes an eggplant produced by this method.

Selection for plants having the combination of parthenocarpic fruit formation and cytoplasmic male sterility can be done in the F1 or any further generation by using molecular markers which directly or indirectly detect the genes underlying the traits. In another aspect selection for the trait of the invention is started in the F2 of a cross or alternatively of a backcross. Selection of plants in the F2 can be done phenotypically as well as by using marker(s) which directly or indirectly detect the genes underlying the traits.
In one embodiment selection for plants having the combination of parthenocarpic fruit formation and cytoplasmic male sterility is started in the F3 or a later generation.

In one embodiment the plant comprising the traits of parthenocarpic fruit formation and cytoplasmic male sterility is a plant of an inbred line, a hybrid, a doubled haploid, or of a segregating population.

The invention further provides a method for the production of an eggplant having the combination of the traits parthenocarpic fruit formation and cytoplasmic male sterility by using a doubled haploid generation technique to generate a doubled haploid line comprising the combination of said traits.

The invention furthermore relates to hybrid seed that can be grown into a plant having the combination of the traits parthenocarpic fruit formation and cytoplasmic male sterility and to a method for producing such hybrid seed comprising crossing a first parent plant with a second parent plant and harvesting the resultant hybrid seed, wherein said first parent plant and/or said second parent plant is the plant as claimed.

In one embodiment, the invention relates to a method for producing a hybrid eggplant that has the combination of the traits parthenocarpic fruit formation and cytoplasmic male sterility, comprising crossing a first parent eggplant with a second parent eggplant and harvesting the resultant hybrid seed, of which the first parent plant and/or the second parent plant has a combination of both parthenocarpic fruit formation and CMS, and growing said hybrid seeds into hybrid plants having a combination of parthenocarpic fruit formation and CMS.

In another embodiment, the invention relates to a method for producing a hybrid eggplant that has the combination of the traits parthenocarpic fruit formation and cytoplasmic male sterility, comprising crossing a first parent eggplant with a second parent eggplant and harvesting the resultant hybrid seed, in which the female parent plant has the trait cytoplasmic male sterility and at least one of the two parents has the trait parthenocarpic fruit formation, and growing said hybrid seeds into hybrid plants having the combination of the traits parthenocarpic fruit formation and cytoplasmic male sterility.

The invention also relates to a method for the production of an eggplant having the combination of parthenocarpic fruit formation and cytoplasmic male sterility by using a seed that comprises the combination of the traits parthenocarpic fruit formation and CMS for growing the said eggplant. The seeds are suitably seeds of which a representative sample was deposited with the NCIMB under deposit number NCIMB 42053.

The invention also relates to a method for seed production comprising growing eggplants from seeds of which a representative sample was deposited with the NCIMB under deposit number NCIMB 42053, allowing the plants to produce seeds, and harvesting those seeds. Production of the seeds is suitably done by crossing.
In one embodiment, the invention relates to a method for the production of an eggplant having a combination of parthenocarpic fruit formation and CMS, by using tissue culture.

The invention furthermore relates to a method for the production of an eggplant having a combination of parthenocarpic fruit formation and CMS by using vegetative reproduction.

The starting materials for tissue culture and vegetative reproduction are suitably plants of the invention, in particular plants grown from seeds of NCIMB 42053 or progeny thereof, or plants that have the CMS of the deposited seeds and another parthenocarp.

In one embodiment, the invention relates to a method for the production of an eggplant having a combination of parthenocarpic fruit formation and CMS by using a method for genetic modification to introgress the said traits into the eggplant. Genetic modification comprises transgenic modification or transgenesis, using a gene from a non-crossable species or a synthetic gene, and cisgenic modification or cisgenesis, using a natural gene, coding for a (agricultural) trait, from the crop plant itself or from a sexually compatible donor plant.

The invention also relates to a breeding method for the development of eggplants that have a combination of parthenocarpic fruit formation and CMS wherein germplasm comprising said trait is used. Representative seed of said plant comprising the combination of parthenocarpic fruit formation and CMS and being representative for the germplasm was deposited with the NCIMB under deposit number NCIMB 42053.

In a further embodiment the invention relates to a method for the production of an eggplant having a combination of parthenocarpic fruit formation and CMS wherein progeny or propagation material of a plant comprising the combination of said traits is used as a source to introgress the combination of said traits into another eggplant. Representative seed of said plant comprising the combination of parthenocarpic fruit formation and CMS was deposited with the NCIMB under deposit number NCIMB 42053.

The invention provides preferably an eggplant having a combination of parthenocarpic fruit formation and CMS, which plant is obtainable by any of the methods herein described and/or familiar to the skilled person.

The invention relates to a cell of an eggplant, which eggplant comprises a combination of parthenocarpic fruit formation and CMS, as found in an eggplant grown from seed as deposited with the NCIMB under the accession number NCIMB 42053.

Another embodiment of the invention relates to a cell of an eggplant, which eggplant comprises a combination of parthenocarpic fruit formation and CMS, as found in an eggplant grown from seed as deposited with the NCIMB under the accession number NCIMB 42053, which eggplant is obtainable by crossing an eggplant with an eggplant grown from seed as deposited with the NCIMB under the accession number NCIMB 42053, and selecting for an eggplant that is capable of parthenocarpic fruit formation and is cytoplasmic male sterile.
Although the cell in itself is not capable of showing the phenotype of the invention it does carry the genetic information that is responsible for the cytoplasmic male sterility and the parthenocarpy, and is as such part of this invention.

The invention also relates to use of seeds that were deposited with the NCIMB under the accession number NCIMB 42053, for transferring the combination of traits parthenocarpic fruit formation and CMS, into another eggplant.

In another embodiment, the invention also relates to the use of an eggplant that comprises the combination of the traits parthenocarpic fruit formation and CMS, as found in an eggplant grown from seed as deposited with the NCIMB under the accession number NCIMB 42053, as a crop.

Furthermore the invention relates to the use of an eggplant which comprises the combination of the traits parthenocarpic fruit formation and CMS, as found in an eggplant grown from seed as deposited with the NCIMB under the accession number NCIMB 42053, as a source of seed.

The invention further relates to the use of an eggplant which comprises the combination of the traits parthenocarpic fruit formation and CMS, as found in an eggplant grown from seed as deposited with the NCIMB under the accession number NCIMB 42053, as a source of propagating material.

Another aspect of the invention relates to the use of an eggplant which comprises the combination of parthenocarpic fruit formation and CMS, as found in an eggplant grown from seed as deposited with the NCIMB under the accession number NCIMB 42053, for consumption.

Furthermore, the invention relates to the use of combined parthenocarpic fruit formation and CMS eggplant alleles as found in eggplant seeds that were deposited with the NCIMB under the accession number NCIMB 42053, for conferring a combination of parthenocarpic fruit formation and CMS, on an eggplant.

Moreover, the invention relates to the use of an eggplant as a recipient of the combined parthenocarpic fruit formation and CMS eggplant alleles as found in seeds that were deposited with the NCIMB under accession number NCIMB 42053.

In a further embodiment the invention relates to a method for selecting a plant having one or more of the parthenocarpic fruit formation and CMS eggplant alleles as found in seeds that were deposited with the NCIMB under accession number NCIMB 42053 comprising screening for the presence of the alleles.

In another embodiment, the invention provides a method for selecting a plant having alleles that confer either or both the traits of CMS and parthenocarpic fruit formation, wherein the alleles are as present in seeds that were deposited with the NCIMB under accession number
NCIMB 42053, wherein the screening is done by optical inspection of flowers for showing non-opening anthers and/or seedless fruits in the absence of fertilization.

Use of alleles that cause parthenocarpic fruit formation and/or CMS for the production of a plant being male sterile and/or producing seedless fruits caused by said alleles, wherein the alleles are as found in seeds that were deposited with the NCIMB under accession number NCIMB 42053.

In the present application the terms "parthenocarp" and "fertilization independent fruit formation" are used interchangeably.

DEPOSIT INFORMATION

Eggplant seeds that carry the combined genetic information for parthenocarpic fruit formation and CMS were deposited on 20 September 2012 with the NCIMB, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, UK under accession number NCIMB 42053.

The invention will be further illustrated in the Examples that follow and that are not intended to limit the invention in any way. In the Examples reference is made to the following figure.

Figure 1: Flowers with non-opening anthers of CMS plants versus flowers with normally opening anthers of non-CMS plants.

EXAMPLES

EXAMPLE 1

Identification of eggplants which have obtained the combination of CMS and fertilization independent fruit formation

Eggplant seeds from an internal line were germinated and were grown into small plantlets. Subsequently, randomly chosen plants were transferred to a greenhouse in which they were raised according to common eggplant cultivation practice. Measures were taken to prevent any insects from outside entering the greenhouse, so cross-pollination between plants was prevented. The eggplants were monitored on a regular basis in order to visually determine which flowers show non-opening anthers which indicates the CMS trait is present in the plant.

Flowers with non-opening anthers of CMS plants versus flowers with normally opening anthers of non-CMS plants are shown in Figure 1. Subsequently, the fruits on the eggplants were monitored on fertilization independent (parthenocarpic) fruit formation. A few criteria were important to select the preferred plants.

The fruits were harvested and opened to check if no seeds had formed and to check whether the fruits were normally filled with fruit flesh. The formation of these fruits without seeds should have a size similar to a normal seeded fruit growing on plants with a similar genetic background. The preferred plants had flowers with non-opening anthers and produced fruits
containing no seeds and having a fruit size and fruit form similar to a normal seeded fruit growing on plants with a similar genetic background.

EXAMPLE 2

Introgression of the combination of the traits of cytoplasmic male sterility (CMS) and parthenocarpic fruit formation

To demonstrate that the combination of CMS and parthenocarpic fruit formation of the invention can be introduced into other eggplant types as well, backcrosses were made with eggplant lines, like the female parent of the variety Nilo RZ F1 as recurrent parent. The plants of the invention, having the combination of traits of CMS and parthenocarpic fruit formation were used as mother plants in these crosses. The resulting F1 and/or F2 progeny produced fruits without fertilization, these fruits contained no seeds and were similar in appearance and characteristics as compared to fruits grown from fertilized flowers of the same plant, under standard Dutch glasshouse conditions.
CLAIMS

1. Eggplant seed capable of growing into a plant that is cytoplasmic male sterile and capable of producing eggplant fruits without fertilization, which seed is obtainable by crossing a mother plant of NCIMB 42053 with a father plant that is optionally parthenocarpic.

2. Plant grown from seed as claimed in claim 1, which plant is cytoplasmic male sterile and capable of producing eggplant fruits without fertilization.

3. Seedless fruit from a plant as claimed in claim 2.

4. Fruit as claimed in claim 3, wherein the fruit does not show negative pleiotropic effects, in particular hollow core and/or deformed shape.

5. Fruit as claimed in claim 3 or 4, wherein the fruit has one or more of the following characteristics:
   a) a size that is similar to the size of a fruit produced after fertilization;
   b) a coloration and ripening process that is similar to the coloration and ripening process of a fruit produced through fertilization.

6. Progeny of an eggplant as claimed in claim 2 that has retained the cytoplasmic male-sterility and capability of fertilization independent fruit formation as found in the parent plant.

7. Propagation material derived from a plant as claimed in claim 2 that has cytoplasmic male-sterility and the capability of fertilization independent fruit formation.

8. Propagation material capable of growing into a plant as claimed in claim 2, wherein the plant has cytoplasmic male-sterility and the capability of fertilization independent fruit formation.

9. Progeny as claimed in claim 6 or propagation material as claimed in claim 7 or 8, wherein the cytoplasmic male sterility is as found in seeds of which a representative sample was deposited under accession number NCIMB 42053.

10. Propagation material as claimed in claim 7 and 8 wherein the propagation material is selected from the group consisting of: microspores, pollen, ovaries, ovules, embryos, embryo sacs, egg cells, cuttings, roots, stems, cells, protoplasts, leaves, cotyledons, hypocotyls, meristematic cells, roots, root tips, microspores, anthers, flowers, seeds, callus and stems or tissue culture or parts thereof.

11. Eggplant fruit, as claimed in claim 3-5, or a food product made of a fruit or made of parts thereof, or a processed food product made thereof, wherein the fruit is harvested from an eggplant as claimed in claim 2.
Figure 1: A. Flowers with normally opening anthers of non-CMS plants. B. Flowers with deformed non-opening anthers of CMS plants.
### INTERNATIONAL SEARCH REPORT

**International application No**

PCT/EP2014/077334

### A. CLASSIFICATION OF SUBJECT MATTER

**INV.** A01H5/08 A01H5/10

**ADD.**

According to International Patent Classification (IPC) and both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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**Date of the actual completion of the international search**  
14 January 2015

**Name and mailing address of the ISA**

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**Authorized officer**  
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