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Kelp

海带

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1 Scope

The document lays down the scientific name and taxonomy of kelp, Saccharina japonica (Areschoug) C.E.Lane, C.Mayes, Druehl & G.W.Saunders, 2006, its main morphological and structural features, its reproductive characteristics, its cytogenetic characteristics and its molecular genetic characteristics; it describes the matching detection methods and lays down the rules for judgement.

It applies to the germplasm testing and identification of kelp.

There are no normative references in the document.

3 Terms and definitions

Sporophyte: the thallus with a diploid chromosome number in the life cycle of kelp. A note states that at maturity it forms sporangia and releases spores.

Gametophyte: the thallus with a haploid chromosome number in the life cycle of kelp, formed by germination of a zoospore. A note states that it is divided into female and male gametophyte.

Length of sporophyte: the total length of the sporophyte, that is the sum of the lengths of the holdfast, the stipe and the blade.

Width of sporophyte: the width of the widest part of the blade of the sporophyte.

4 Scientific name and taxonomy

The scientific name is Saccharina japonica (Areschoug) C.E.Lane, C.Mayes, Druehl & G.W.Saunders, 2006.

Its taxonomic position is phylum Ochrophyta, class Phaeophyceae, order Laminariales, family Laminariaceae, genus Saccharina.

5 Main morphological and structural features

5.1 The sporophyte is made up of holdfast, stipe and blade. The holdfast consists of several layers of cylindrical, dichotomously branched haptera. The stipe is cylindrical or flattened cylindrical, yellowish brown to dark brown, and in the adult plant is 3 cm to 13 cm long. The blade is strap-shaped, brown and glossy, its base is wedge-shaped when young and close to rounded at the adult stage. The blade grows thinner from the midline towards the margin, and the margin is thinner and softer than the middle band and is undulate and folded; longitudinal grooves may be absent or present, and where they are present two shallow grooves run through the middle of the blade and mark out a distinct middle band. At maturity, pale yellow strips or patches rise on the surface of the blade, which are the sori. Adult wild kelp is generally 0.3 m to 1.8 m long and 4 cm to 20 cm wide; adult cultivated kelp is 2 m to 9 m long and 20 cm to 90 cm wide. The outward form of the sporophyte is shown in Figure 1, whose key numbers the holdfast, the stipe, the blade, the longitudinal groove, the middle band, the marginal part, the length and the width.

5.2 The epidermis is made up of small, closely and regularly arranged cells holding granular chromatophores, set out like a palisade. In the cortex, the cells of the outer cortex are columnar and of unequal size and are not regularly arranged, while the cells of the inner cortex are close in size and more regularly arranged. The medulla is made up of trumpet hyphae and medullary filaments.

6 Reproductive characteristics

6.1 The life cycle is an alternation of unequal generations between the sporophyte, which is diploid, and the gametophyte, which is haploid. The life cycle is shown schematically in Figure 2.

6.2.1 Wild kelp reproduces from March to June and from September to December each year. Cultivated kelp reproduces from May to August, and under indoor culture up to December.

6.2.2 In asexual reproduction the sporophyte forms unilocular sporangia in sori and produces zoospores. The zoospore is unicellular and pear-shaped, 6.9 μm to 8.2 μm long and 4.1 μm to 5.5 μm wide, with two laterally inserted flagella of unequal length, the long flagellum 17.8 μm to 19.6 μm and the short flagellum 6.9 μm to 8.2 μm long. The form of the

zoospore is shown in Figure 3.

6.2.3 Sexual reproduction is oogamous. The female gametophyte forms oogonia, each producing one yellowish brown egg. Once ripe the egg is released from the oogonium and sticks to its outer wall; it is oval and 8 μm to 10 μm across. The male gametophyte forms antheridia, each producing one pear-shaped sperm. The sperm is 4.4 μm to 6.2 μm long and 2.7 μm to 3.4 μm wide, with two laterally inserted flagella of unequal length, the short flagellum 13.7 μm to 16 μm and the long flagellum 16 μm to 19 μm long. The haploid sperm and the haploid egg join to give a diploid zygote. The forms of the female and male gametophytes are shown in Figure 4.

7 Cytogenetic characteristics

The chromosome number is n = 31 and 2n = 62.

8 Molecular genetic characteristics

A reference sequence of the mitochondrial COI gene fragment, of 609 base pairs, is given, and the within-species genetic distance calculated by the Kimura two-parameter model shall be less than 2 %. The reference sequence itself is not reproduced here: it did not come through in the extracted text of the document.

9 Detection methods

9.1.1 The outward form is detected by laying the sporophyte out flat and examining it by eye in natural light.

9.1.2 For the internal structure, tissue is taken from the part to be examined at a thickness of 0.5 mm to 1 mm, a suitable amount of seawater is dropped on it, a cover glass is laid over it and it is examined under the microscope.

9.2.1 The time at which the sporophyte forms sori is taken as the time of reproduction. The sori are observed by eye in sufficient light.

9.2.2 The forms of the zoospores, the female and male gametophytes, the eggs and the sperm are examined under the microscope.

9.3 The cytogenetic characteristics are detected by the method of Annex A.

9.4 The molecular genetic characteristics are detected by the method of Annex B.

10 Rules for judgement

10.1 Where the detection result meets the requirements of clause 5, the material is judged to be of the species.

10.2 In the following cases the content of further clauses shall also be detected and the species judged on the whole of the detection results: where clause 5 cannot be detected or does not allow an accurate judgement, the content of clause 7 or clause 8 is detected in addition; where a third party asks for all or part of the content of clause 6 to be detected; and where all items are to be detected.

A Annex A (normative) Detection of the cytogenetic characteristics of kelp

A.1 Solutions are prepared as follows. Colchicine: 0.1 g of colchicine is made up with 10 mL of distilled water into a 1 % stock solution, kept between 2 °C and 8 °C away from light and used within 2 weeks, and diluted with distilled water to the concentration needed at the time of use. Carnoy's fixative: glacial acetic acid and absolute ethanol are poured in a volume ratio of 1 to 3 into a prepared solution bottle and stirred gently with a glass rod until even; it is kept at room temperature away from light and used within 2 weeks. Mixed enzyme solution: cellulase, pectinase, macerozyme and abalone enzyme in the ratio 2 to 1 to 1 to 1.5. DAPI stain: dry 4',6-diamidino-2-phenylindole powder is dissolved in distilled water to a stock solution of 5 mg/mL, and diluted with phosphate buffered saline at pH 7.0 in a volume ratio of 1 to 1 000 to give the working solution; the stock is kept at -20 °C away from light, the working solution at 4 °C away from light, and both are used within 2 weeks.

A.2 A suitable amount of the kelp material is washed two or three times with sterilised seawater; surface water is blotted from sporophyte material with absorbent paper and removed from gametophyte material by centrifuging. Colchicine at 0.2 % is added, the material shaken to mix and held at 4 °C for 6 h to 12 h. The gametophyte pellet is taken by centrifuging and the sporophyte material lifted out directly with tweezers; Carnoy's fixative is added, the material shaken to mix, held at 4 °C for more than 24 h, then washed three to five times with distilled water for 2 min each time. The gametophyte pellet is again taken by centrifuging and the sporophyte material lifted out with tweezers, put into a centrifuge tube with a suitable amount of the mixed enzyme solution and digested for 18 h at 37 °C on a shaker at 200 r/min. The digestion is stopped with 70 % ethanol, the digest washed three or four times with distilled water, a suitable amount of glacial acetic acid kept at 4 °C is added and the material shaken to mix; the digest is checked for evenness, a small amount is dropped onto a slide and the slide is left to dry in air.

A.3 A slide carrying chromosomes and dried in air is stained with 15 µL of DAPI for 15 min, then observed and photographed under a fluorescence microscope with a 100 times objective.

B Annex B (normative) Detection of the molecular genetic characteristics of kelp