



Circumnutation on the water surface: female flowers of *Vallisneria*

Keiko Kosuge¹, Satoko Iida¹, Kiyoshi Katou² & Tetsuro Mimura¹

¹Department of Biology, Graduate School of Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan, ²Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan.

SUBJECT AREAS:
PLANT PHYSIOLOGY
TROPISM
BEHAVIOURAL ECOLOGY
PLANT ECOLOGY

Received
7 August 2012

Accepted
18 December 2012

Published
25 January 2013

Correspondence and
requests for materials
should be addressed to
K.K. (kosuge@kobe-u.
ac.jp)

Circumnutation, the helical movement of growing organ tips, is ubiquitous in land plants. The mechanisms underlying circumnutation have been debated since Darwin's time. Experiments in space and mutant analyses have revealed that internal oscillatory (tropism-independent) movement and gravitropic response are involved in circumnutation. Female flower buds of tape grass (*Vallisneria asiatica* var. *biwaensis*) circumnutate on the water surface. Our observations and experiments with an artificial model indicated that gravitropism is barely involved in circumnutation. Instead, we show that helical intercalary growth at the base of peduncle plays the primary role in all movements in *Vallisneria*. This growth pattern produces torsional bud rotation, and gravity and buoyancy forces have a physical effect on the direction of peduncle elongation, resulting in bud circumnutation on the water surface. In contrast to other water-pollinated hydrophilous plants, circumnutation in *Vallisneria* enables female flowers to actively collect male flowers from a larger surface area of water.

*"As dash the waves on India's breezy strand,
Her flush'd cheek press'd upon her lily hand,
VALLISNER sits, up-turns her tearful eyes,
Calls her lost lover, and upbraids the skies"*¹

Erasmus Darwin noting that the ingenious pollination method of *Vallisneria spiralis* resembled the reproductive behaviour of some insects, such as the firefly and winter moth, in which the males, but not the females, develop wings during the mating season¹.

Rapidly elongating plant organ tips, such as roots, hypocotyls, shoots, branches, and peduncles, often show helical or pendulum-like movements. Charles Darwin and his son termed these movements "circumnutations" and proposed that they were driven by an internal oscillatory (tropism-independent) movement, and were modified by tropisms of exogenous factors such as gravity or light². Subsequently, the importance of gravity, which induces the differential cellular/tissue growth of gravitropic bending zone (gravitropic response), was emphasized³. Experiments in space, however, revealed that both endogenous and exogenous factors induced circumnutations. In conditions of microgravity, the growing tips of sunflower seedlings and lateral shoots of *Arabidopsis thaliana* exhibited small nutational movements, the amplitude of which were increased by gravitational acceleration^{4,5}. For example, the amplitude of oscillations in 4- to 5-day-old sunflower hypocotyls was 7.36 mm on Earth and 2.77 mm under microgravity conditions⁴. Mutant analyses also indicated that the gravitropic response was important for circumnutation. Agravitropic mutants lacking starch-filled amyloplasts in endodermal cells were defective in shoot circumnutation in morning glory and *Arabidopsis*^{6,7}. At present, circumnutation is presumed to depend upon the internal oscillatory movement and gravity sensing^{4,5,8}. However, other stimuli, such as light, temperature, and mechanical stress, also affect circumnutation⁹. Further, reversible cellular volume changes¹⁰, orientation of microtubules¹¹ and the circadian clock^{12,13} were reported to be involved. The innate nature of the gravitropic response in land plants makes it difficult to identify whether and how the internal oscillatory movement, tropism, and exogenous forces are involved in circumnutation. Shoot movements in an aquatic environment are interesting because buoyancy diminishes the effect of gravity. For example, submerged rice coleoptiles, which contain few amyloplasts, displayed rapid and circumnutation-like growth¹⁴.

Vallisneria is a monocot genus of dioecious species (Hydrocharitaceae) that grow submersed at the bottom of freshwater bodies and undergo hydrophilous pollination on the water surface^{1,15-18} (Fig. 1a, see also Supplementary Fig. S1). A single female flower develops underwater and is brought to the surface by elongation of its slender



peduncle. At anthesis, it floats on the water surface. Male flower inflorescences are produced underwater. At maturity, the male flower buds detach and rise to the water surface where the three sepals open to expose the stamens. The male flowers are dispersed across the water surface and aggregate around a female flower, and pollination is effected by contact between a stamen and the stigma. After pollination, the peduncle of the female flower coils up tightly and retracts the pollinated flower underwater. Although Darwin and Sachs considered coiling of the *Vallisneria* peduncle to be similar to that of the tendrils of many climbing plants^{19,20}, they did not report the resulting circumnutation of its female flower-bud on the water surface.

Recently, Gerbode and colleagues investigated the mechanical behaviour of helical coiling of cucumber tendrils²¹. These authors elucidated biological and physiological mechanisms for the transformation of a straight tendril into a helical structure after it is tethered to a support. However, the mechanism underlying the circular movement of straight tendrils in search of a support is still obscure. Here, we describe the movements during development of female

flowers of *Vallisneria asiatica* var. *biwaensis* Miki. In addition, to investigate whether gravitropism or other biological sensing systems for environmental stimuli are involved in the circumnutation of *Vallisneria*, we conducted experiments with an artificial model of a female flower bud and peduncle.

Results

Movements of female flowers. We tracked the movements of female flowers using time-lapse video images (Fig. 1, Supplementary Movies 1 and 2). The floral bud peduncle elongated upwards underwater while rotating in a counter-clockwise direction when viewed from above (stage 1: Fig. 1b and 1c-1). The bud rotated about its central axis similar to a turn screw (torsional rotation) and the slender peduncle gently twisted into a right-handed helix (Fig. 1c-1, Fig. 2a).

The floral bud was unable to be held on the peduncle above the water surface against gravity. Therefore, once the bud reached the water surface, the upper part of the peduncle bent and the floating bud turned on the perpendicular axis from the base of peduncle, that is, circumnutation occurred (stage 2: Fig. 1b and 1c-2). During the

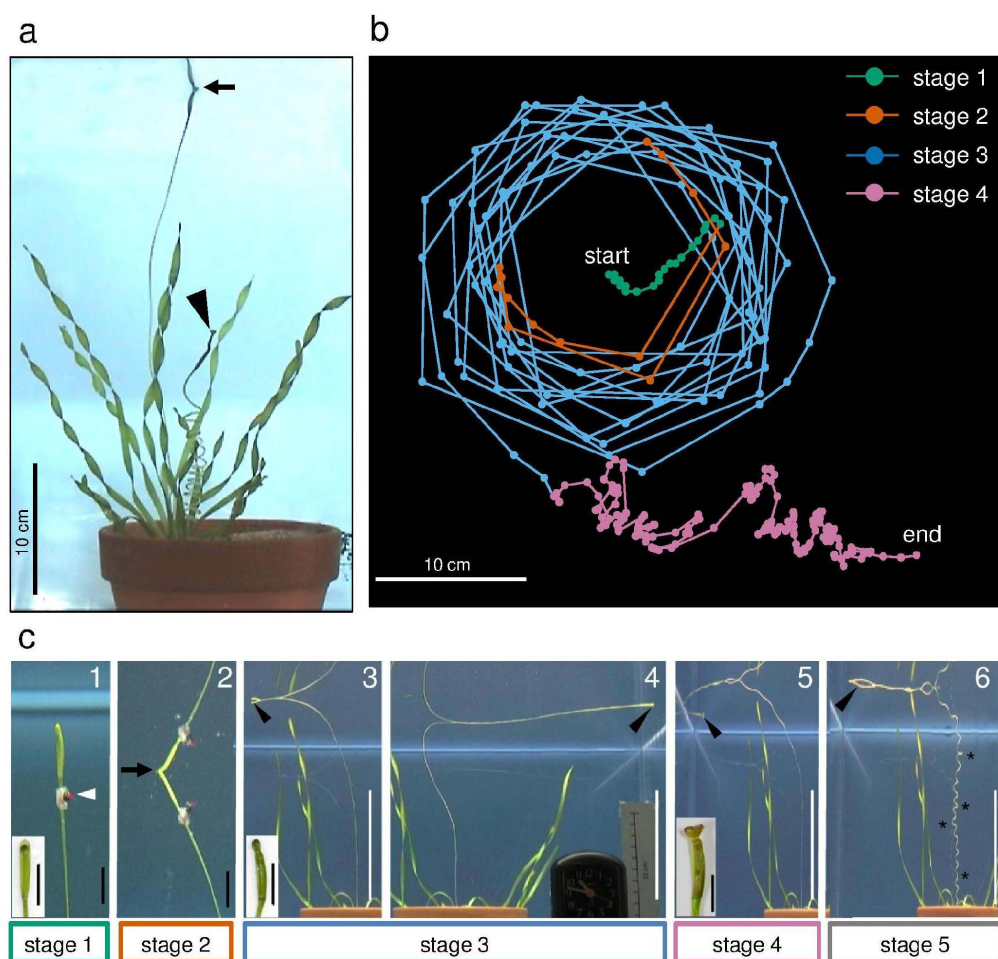


Figure 1 | Typical movement of *Vallisneria* female flower in shallow-water tank. (a) Side-view of *Vallisneria asiatica* var. *biwaensis* plant with two female flowers: bud extending to the water surface (arrow) and developing fruit with coiled peduncle underwater (arrowhead). Reflection on the water surface forms a mirror image of the plant. Leaf blade twists into a left-handed helix, where epidermal cells of the marginal portion become relatively longer than those along the midvein and are deformed during development (Okamoto, M. personal communication). (b) Trajectory of bud tip extracted from Supplementary Movie 1. Line colours reflect differences in bud movement. Stage 1: rotation underwater; stage 2: circumnutation on water surface; stage 3: circumnutation and rotation on water surface; stage 4: rotation and disturbance of circumnutation on water surface. Trajectory was recorded at 5-min intervals. (c) Side-view images obtained from Supplementary Movie 2. Definition of stages 1–4 is the same as in (b). Stage 5 shows retraction of developing fruit underwater by coiled peduncle after pollination. Insets show flower bud (1, 3) and flower (5). Silicone tube collar with a plastic ball was attached near the bud base (white arrowhead) to monitor stalk rotation. Arrow: water surface, black arrowhead: bud or flower. Scale bars, 10 mm (1, 2), 10 cm (3–6), 10 mm (1, 3, 5, inset). Asterisk: perversion. Reflection on the water surface forms a mirror image in images 2–6.

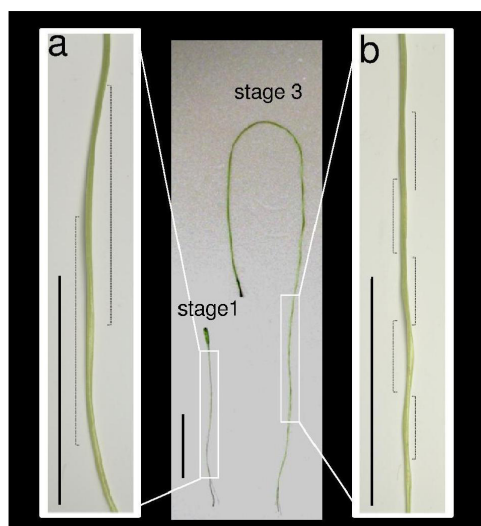


Figure 2 | Flower-stalk rotation in *Vallisneria*. Compared with peduncle underwater (stage 1), stalk is well twisted after circumnavigation for 1 day at stage 3 (helix pitch, (a): 3.38 ± 0.60 cm, $n = 3$ and (b): 1.32 ± 0.98 cm, $n = 3$) except near the bud. Each square bracket indicates a single pitch of one rotation. Scale bars, 3 cm. After excised peduncles were deposited horizontally in shallow water for 1 day, helical structures have become loose (changed helix pitch, (a): 6.80 ± 0.77 cm and (b): 1.96 ± 0.34 cm).

circumnutation in stage 2, the bud tilted to the left and right but did not rotate; therefore, the peduncle increased the number of helices. Thereafter, rotation was reinitiated and the bud exhibited both rotation and circumnutation (stage 3: Fig. 1b and 1c-3) and the peduncle became more twisted after circumnavigation for 1 day (Fig. 2b). As the peduncle elongated, the bud and upper part of the peduncle floated on the water surface (Fig. 1c-4). The trajectory of the circumnutation initially formed an arc shape and frequently changed in direction (stage 2: Fig. 1b, Fig. 3a), then became circular and was frequently in a counter-clockwise direction (stage 3: Fig. 1b, Fig. 3b). Further

elongation of the peduncle hindered circumnutation but rotation continued (stage 4: Fig. 1b and 1c-5).

After pollination, both elongation of the peduncle and rotation of the flower ceased and the peduncle was wound into loose helical coils (stage 5: Fig. 1c-6). Subsequently, the peduncle began to coil from the base similar to an extended spring. As a result, the developing fruit was withdrawn below the water surface (Fig. 1a). The peduncle coil was mostly in a counter-clockwise direction but spontaneously changed in direction (Fig. 1c-6, Fig. S1–e), causing switching of the helical structure from one handedness to its mirror image, which is termed perversion^{19,21,22}.

Anatomy of female flower peduncle. We examined the peduncle anatomy and the changes therein during stages 1–5. Air was lost from within the peduncle and the helical structure had become loose during fixation with FAA or 70% ethanol. Therefore, excised fresh samples were immediately cut into sections or soaked in clearing solution. The peduncle was elliptical in cross-section. Two sets of vascular bundles lacking a parenchymatous sheath were arranged asymmetrically (Fig. 4). One vascular bundle was centrally positioned in the cross-section and surrounded by cortex tissue with large air spaces (aerenchyma); the other vascular bundle was laterally offset and surrounded by more densely packed cortical cells with only small air spaces.

At the onset of rotation (stage 1), well-developed air spaces were present in the flower bud and the upper part of the peduncle (Fig. 4a). More and larger air spaces, which were surrounded by cortex cells and a transverse diaphragm cell layer, were formed around the central bundle than around the lateral bundle in the peduncle (Fig. 4b–e). At the onset of circumnutation on the water surface (stage 2), the vertical files of spaces within the aerenchyma tissue were obliquely oriented (Fig. 4f) along the helix of the peduncle (Fig. 2a). Air spaces in the peduncle were notably compressed during the circumnutation (Fig. 4g–i). At stages 3 and 4, the peduncle was so strongly twisted that we could not obtain clear sections of fresh material (Fig. 2b, Fig. 4j).

After pollination (stage 5), the peduncle was swollen along most of its length and had become strongly coiled (Fig. 4k). The coiled

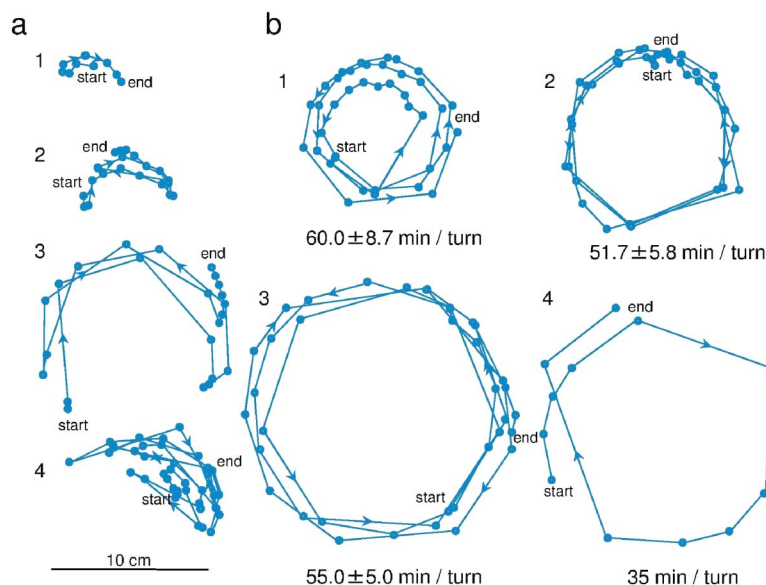


Figure 3 | Circumnutation trajectories of *Vallisneria* female flowers on water surface. Images of four bud tips (1–4) were extracted from movies. Trajectory line obtained for each bud is drawn separately in stages 2 and 3. (a) Zig-zag and pendulum-like circumnutations after reaching water surface (stage 2). (b) Circular circumnutations during stage 3. Numerical values indicate time required for a single circumnutation, and represent average value \pm SD for buds (1–3) and single bud circumnutation (4). Each point indicates tip position at 5 min intervals. Arrowhead shows direction of movement. Flowers moved in a counter-clockwise direction more frequently than in a clockwise direction.

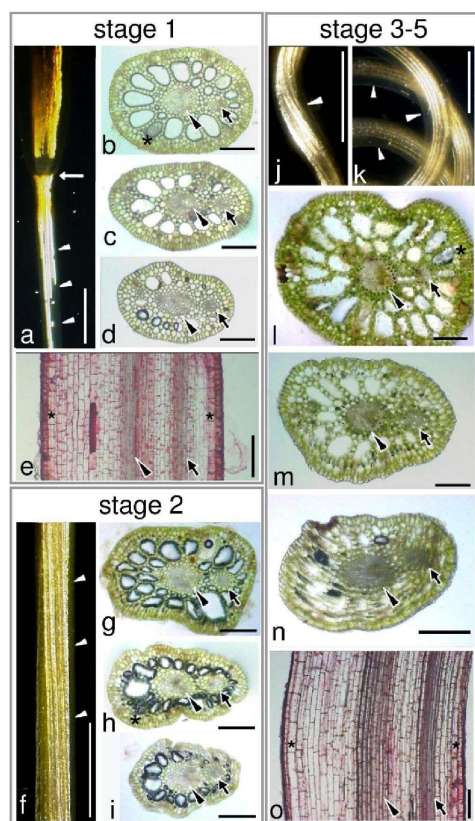


Figure 4 | Anatomy of *Vallisneria* female flower peduncle.

(a, f, j, k) Peduncle cells become translucent after chloral hydrate treatment and files of air spaces within aerenchyma tissues shine brightly (scale bars, 2 mm). Arrowheads indicate translucent peduncle surface near lateral bundle. (a) Air spaces develop in flower bud and upper part of the peduncle at the onset of rotation (stage 1). Arrow indicates boundary between flower bud and peduncle. (f) At onset of circumnutation (stage 2), air spaces are filled with air and their files are obliquely oriented in the peduncle.

(j) Files of air spaces wind along the helix of the peduncle during stage 3 to 4. (k) After pollination (stage 5), peduncle becomes coiled. Air spaces along inside of coil (near the lateral bundle, arrowheads) disappear and fill with sticky substances, while those along outside of coil (near the central bundle) remain in files. Cross-sections of upper (b, g, l), central (c, h, m), and lower (d, i, n) portions of peduncle (scale bars, 20 μm). Central vascular bundle tissue (arrowhead) is larger than lateral one (arrow). Diaphragm cells, a transverse cell layer of aerenchyma tissue with very small pores, develop during stage 1–4 (b, h; asterisk), but collapse during stage 5 (l; asterisk). (e, o) Longitudinal sections of central portion of a peduncle stained with safranin-alcohol (scale bars, 20 μm).

(e) Hypodermal cells (outermost cell layer of cortex, asterisks) are almost identical in length between outside ($9.58 \pm 1.57 \mu\text{m}$) and inside ($9.39 \pm 1.86 \mu\text{m}$) of the peduncle (stage 1, $n = 20$, $P = 0.76$; t-test).

(o) Hypodermal cells along outside of peduncle coil ($22.73 \pm 2.27 \mu\text{m}$) are 1.5 times longer than those on inside ($15.17 \pm 3.19 \mu\text{m}$) (stage 5, $n = 15$, $P < 0.001$; t-test).

structure was irreversible as compared with the helical structure during stages 1–4 (see legend in Fig. 2). Along the inside of the coil, where the lateral bundle was located, air spaces had become partially filled with mucilage (Fig. 4k and 4m). Near the flower base, the peduncle had become spherical in cross-section and the characteristic asymmetrical anatomy observed in previous stages had almost disappeared (Fig. 4l). In the basal part, the cell files were strongly skewed in a right-handed direction (Fig. 4n). In longitudinal view, cells in the hypodermal layer on the outside of the coil were about 1.5 times longer than hypodermal cells on the inside (Fig. 4o).

Interaction between growth and environmental factors. To determine whether circumnutation is a characteristic behaviour on the water surface, we cultivated plants in a deep-water tank (Fig. S2). The bud–peduncles with a single silicone tube collar continued rotation for more than 72 hours without circumnutation (Fig. S2a). The time per rotation of a bud ($2.41 \pm 0.58 \text{ h}$) and the speed of peduncle elongation ($0.93 \pm 0.29 \text{ cm h}^{-1}$) were relatively constant. To observe basal intercalary growth, seven silicone tube collars were attached along a young peduncle (Fig. S2b). Similar to terrestrial grasses, elongating intercalary tissues are located in the basal region of the peduncle of *Vallisneria* (Fig. S2b–e). Consequently, we hypothesized that helical growth of the peduncle creates the bud rotation that generates the driving force for circumnutation on the water surface.

We performed experiments with an artificial model to examine further the relationship between rotation and circumnutation (Fig. 5, Fig. S3, Supplementary Movie 3). Rotation of an artificial flower bud (balsa wood sheet) and peduncle (silicone tube) was generated using a low-speed magnetic stirrer and peduncle elongation on the water surface was simulated by draining water during the experiments. However, the artificial bud–peduncle model could not mimic the spiral or twisting structure of the *Vallisneria* peduncle, because the silicon peduncle was elastic and was not clamped at the base. Neither elongation nor rotation alone induced circumnutation (Supplementary Movie 3). Underwater, the balsa bud rotated at the stirring speed. When the water level decreased and the silicon peduncle was bent by gravity, the torsion movement of the peduncle induced the balsa bud to circumnutate rather than rotate (Fig. 5, Fig. S3b). During the first and second circumnutation, the balsa bud tip moved in a circular path at the stirring speed (Fig. 5a and 5b). When the silicon peduncle bent further, the circumnutation increased in amplitude and became slower, then, the rotation of the balsa bud was reinitiated (Fig. 5b, fourth and eighth circumnutations). The rotations increased in number as the circumnutation cycle proceeded, i.e., 0.75 ± 0.96 rotations observed in the third circumnutation, 2 ± 0.82 in the fourth, and 4 ± 0.82 in the eighth ($n = 4$; e.g., Fig. 5a).

The trajectory of the balsa bud tip showed that rotational and circumnutational movements were negatively correlated; that is, when circumnutation was reduced, the bud rotation accelerated (indicated by * in Fig. 5a). As the balsa bud began to rotate slowly, the silicon peduncle became distorted underwater and shortened. Thereafter, the balsa bud rapidly turned and the silicon peduncle straightened. After the balsa bud briefly ceased movement, the next circumnutation and rotation of the bud started again. Given that the *Vallisneria* peduncle is less elastic than that of the artificial model, the transitions in peduncle length were obscured in the upper view (Fig. 3). However, the peduncle distortion underwater and the irregular bud circumnutation and rotation on the water surface were observed in the side view (Fig. 1c–4).

Discussion

Rotating and twisting movements of rapidly elongating plant organs have long fascinated biologists and have been described in a variety of land plants^{2–14}. In the present study, we demonstrated the occurrence of circumnutation in the aquatic plant *Vallisneria asiatica* (Fig. 1, Fig. 2). Observations (Fig. 1–4) and experiments with an artificial model (Fig. 5) revealed that helical intercalary growth of the peduncle is “an internal oscillator” that drives the circumnutations on the water surface in the absence of biological sensing systems for environmental stimuli.

Circumnutation is widely ascribed to differential elongation or expansion along opposite sides of the growing organs such as shoots and roots (i.e. tubular organs). Polar auxin transport is involved in this process. In *Arabidopsis* roots, a gravity stimulus causes the asymmetric release of auxin from gravity-sensing columella cells at the root cap toward the detached elongation zone²³. The gravitropic

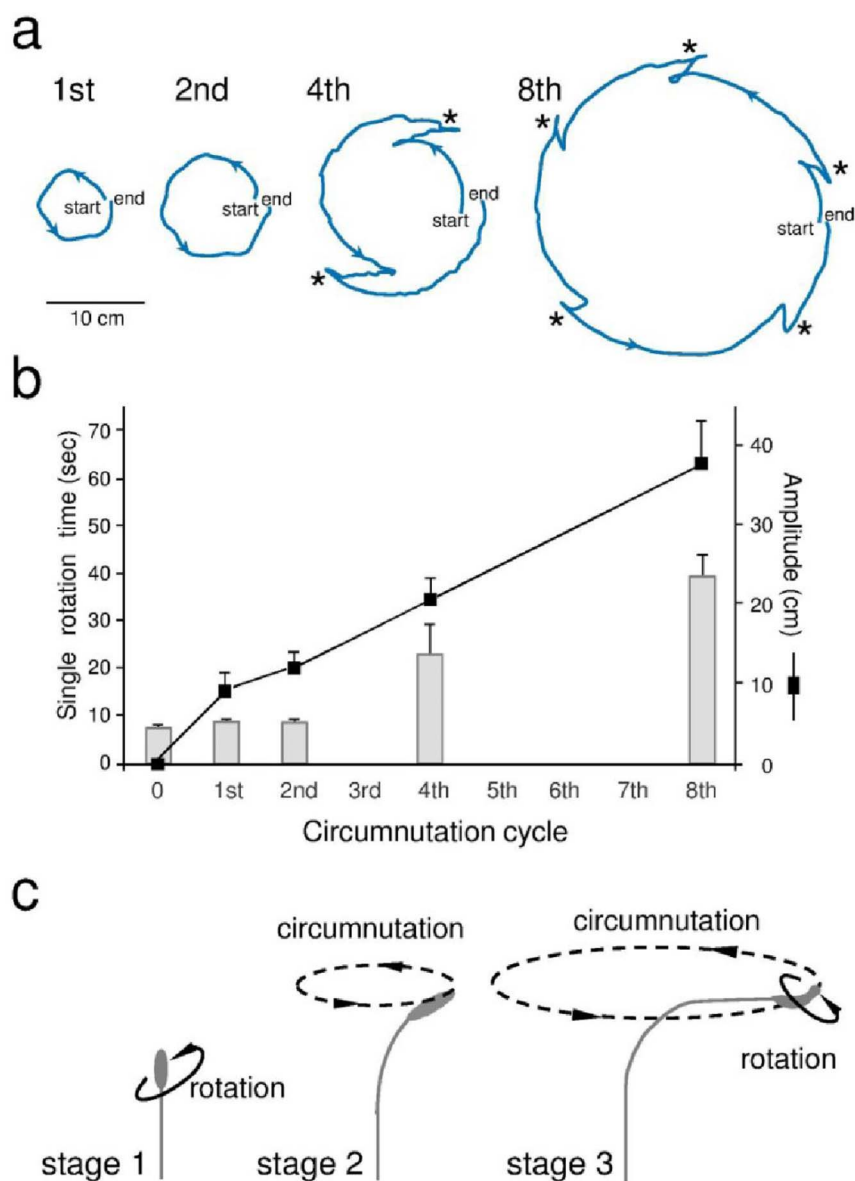


Figure 5 | Movement of helically growing artificial bud-peduncle model on water surface. (a) Typical example of trajectories of an artificial bud tip on water surface. Trajectories for first, second, fourth, and eighth circumnutational cycles are shown separately. Direction of movement is indicated by arrowhead. Counter-clockwise rotation of artificial bud occurred during fourth and eighth circumnutational cycles. Start points of each rotation are indicated by asterisks. (b) Time required for a single circumnutation (single rotation time) and amplitude of circumnutation of an artificial bud on water surface. At circumnutation cycle 0, single rotation time is the rotation speed of magnetic stirrer used to generate helical movement of artificial bud-peduncle model. Each bar indicates average value + SD of four independent experiments. (c) Schematic illustrations of movements of a *Vallisneria* female flower (stages 1–3 in Fig. 1).

bending is primarily driven by auxin-mediated differential expansion of epidermal cells, which modify the growth pattern of the underlying cortical and endodermal tissues²³. In the shoot, the gravity signal is perceived in the endodermal cell layer²⁴. Although a number of studies suggested that auxin may act in differential cell elongation, no distinct signaling pathway is known for shoot gravitropism²⁵. Circumnutation of various plants cannot be explained solely by differential growth. In *Phaseolus vulgaris*, the revolving movement of the twining shoot is driven by reversible and turgor-mediated volume changes in the bending-zone cells¹⁰. At present, researchers are investigating the direct regulation of cell expansion using helical growth mutants. In helical growth mutants of *Arabidopsis*, inflorescence stems showed twining behaviour similar to that of the tendrils of climbing plants¹¹. The twisting behaviour of *Arabidopsis spr1* mutants, which show obliquely oriented cortical

microtubules in elongating cells, has been explained as the result of highly reduced longitudinal expansion of the cortex and endodermis cells, which caused skewed anisotropic growth of epidermal cells and generation of helical growth of the stem^{26,27}. However, an alternative explanation was proposed for twisting in this mutant, namely that a radial gradient in cell elongation and angle towards the organ axis must occur in twisting organs because of geometric constraints but not differential molecular expression²⁸. This means that when epidermal cells are skewed, geometric constraints will lead to tilting of the underlying cells and the organ will adopt a helical structure.

In the Hydrocharitaceae, the single-flowered inflorescence is thought to be derived from the fusion of pedicels and the loss of late-formed flowers²⁹. The asymmetrical arrangement of the two vascular bundles within the peduncle occurs in both *Vallisneria* (Fig. 4) and *Enhalus*^{30,31}. Based on anatomical studies in both genera,



Svedelius noted that a coiling of the peduncle might be caused by different growths of the two bundles³⁰. In this study, we could not identify a different growth pattern between the two bundles, or on opposite sides of the peduncle during stages 1–4 (Fig. 4f and 4j). Brown suggested that if a small asymmetry developed, the organ will bend³². Consequently, the peripheral tissues on the convex side of the bend are stretched and those on the concave side are compressed. In *Vallisneria asiatica*, the asymmetrical development of air space files and two bundles may be important for creating the helical structure of the peduncle (Fig. 2). In addition, the buoyancy of the air-filled bud may assist with rapid intercalary growth (Fig. S2). As a result, the peduncle grows helically and the bud rotates torsionally underwater (stage 1, Fig. 5c). The helical structure is partially reversible during rotation and circumnutation, but becomes irreversible after differential cell elongation (Fig. 4o), and consequently the peduncle becomes tightly coiled (stage 5, Fig. 1c–6). Recent investigations on tendrils and twining vines suggested that a cylinder of cortical gelatinous (G) fibres, which are enriched in acidic polysaccharides, generate a contractile force to transform elongated tendrils into highly coiled structures^{33,34}. In cucumber, helical coiling of the tendril occurs via asymmetrical lignification and contraction of internal G fibre cells after the tendril is tethered to a support²¹. Although specialised lignified cells were not observed in the *Vallisneria* peduncle (Fig. 4l–n), the helical arrangement of vascular bundles and peduncle cells, the differential growth of hypodermal cells and replacement of air in the aerenchyma tissues by mucilages (Fig. 4j, 4k, 4o) might play roles in the rapid coil formation after pollination.

Circumnutation in *Vallisneria* occurs only on the water surface, where the direction of peduncle elongation must be changed by the effect of the force of gravity. Consequently, the bud floats and the rotational force generates the bud circumnutation on the water surface (stage 2, Fig. 5c). The speed of bud rotation and circumnutation is altered by the direct interaction with the water drag force, as observed in the artificial bud–peduncle model (stage 3, Fig. 5c). At the onset of circumnutation, complex forces (such as forces generated by helical elongation of the peduncle, buoyancy, and water drag) acted on the bud, which caused the chaotic movement of the bud (stage 2, Fig. 1b and 1c–2, Fig. 3a). Compared with the artificial-model experiments, the speed of *Vallisneria* circumnutation is faster than that of bud rotation underwater. In the artificial-model experiments, circumnutation could directly release the force generated by stirring, because the artificial peduncle was free from the base. In contrast, the *Vallisneria* peduncle, which is fixed at the base, must create a new helix after each cycle of circumnutation. During the circumnutation, the peduncle became more twisted (Fig. 2b), and consequently the rotational force increased compared with that during underwater bud rotation (Fig. 2a). The additive effect on total force may accelerate the rotation as well as the circumnutation, although we could not measure the rotation speed during circumnutation. Furthermore, *Vallisneria* plants contain terpenes and phenolic compounds, which are released into the surrounding water^{35,36}. As small particles moved on the water surface (Supplementary Movies 1, 2), we hypothesize that released chemical compounds decrease the surface tension of the water and increase the mobility of floating plant bodies. As demonstrated in the artificial-model experiment, the water drag increased during elongation of the peduncle on the water surface, which resulted in reinitiation of bud rotation and reduction of circumnutation (stages 3 and 4, Fig. 1c–4 and 1c–5, Fig. 5c).

The functional importance of circumnutation for supporting the shoot of climbing plants and tendrils is obvious^{2,19,20}. Root-tip rotation is also advantageous in terms of soil penetration for seedling establishment³⁷. The ecological value of helical structure of the *Vallisneria* peduncle includes ensuring flotation of the flowers on the fluctuating water surface, and retraction and anchoring of developing fruits underwater³⁸. However, it is not so clear what the

functional value of circumnutation of the *Vallisneria* flower is. For *Vallisneria* and other hydrophilous plants, it was long thought that the creation of a surface-depression by the female flower enabled better capture of male flowers transported by waves or wind currents^{1,15–18,39}. In contrast, field studies of *Vallisneria americana* revealed that fruit set was negatively correlated with surface water velocity and, compared with open-water areas, fruit set increased in sites protected from waves and wind, even though male flowers were abundant³⁹. Our observations can resolve this contradiction; circumnutation of female flowers generates waves and aggregates male flowers floating on the water surface.

Whether the mechanism underlying circumnutation is the internal oscillatory movement and/or the involvement of tropisms (sensing) has long been debated. The present results show that female flowers of *Vallisneria* can circumnutate in the absence of gravitropism. The helical growth of the peduncle plays the primary role in all movements, and the forces of gravity and buoyancy affect only physically the direction of peduncle elongation, resulting in the bud circumnutation on the water surface. In *Vallisneria*, the circumnutation is indispensable for hydrophilous pollination on the water surface and creation of a coiled structure with which to retract the fruit underwater.

Methods

Plant movement. Plants of *Vallisneria asiatica* var. *biwaensis* were collected from Lake Biwa, Shiga Prefecture, Japan, and cultivated in experimental ponds at Kobe University. For observations of circumnutation movement, plants were cultivated in a shallow-water tank (50 cm³ acrylic tank, 25 cm in depth from the water surface to the base of the plants) or in a deep-water tank (W × L × H = 35 × 15 × 200 cm, 150 cm in depth from the water surface to the base of the plants) at room temperature (25°C). Plants were grown under continuous lighting supplied by fluorescent lamps (FL20SSW/18, FL20SFRR/20, Panasonic, Osaka, Japan). The irradiation intensity was 8–20 μmol photons m⁻² s⁻¹ at the water surface in the shallow-water tank. Irradiance in the deep-water tank was 17 μmol photon m⁻² s⁻¹ at the base of the plant (measured in the absence of water). Leaves were partially clamped to the soil using paper clips. To observe movement after pollination (stage 5), a female flower was pollinated by the addition of male flowers to the tank 9 h after flowering. All images in the supplemental movies were recorded with a time-lapse video camera (HDR-HC1, Sony, Tokyo, Japan). Image stacks were recorded every minute. Movies were recorded in two directions: side-view ($n = 8$ in the shallow-water tank, $n = 7$ in the deep-water tank) and upper-view ($n = 5$). To observe peduncle movement underwater, a notched silicone tube (inside diameter 1 mm, outside diameter 2 mm) collar with a plastic ball was attached near the bud base using silicone grease. Images were resolved from the movies at five-minute intervals to determine the trajectory of the bud or flower tip. The width of one complete helical turn (the pitch) was measured from a magnified photograph of the peduncle.

Excised peduncles were immediately embedded in a 5% agar block (in a hole bored with a hematocrit tube). The agar block containing the peduncle piece was fixed to the sample tray with superglue and cut into sections (50–100 μm thick) in water using a Linear Slicer Pro 7 (Dosaka EM, Kyoto, Japan). Air bubbles in the aerenchyma tissue of sections were removed by gentle pipetting, and sections were observed under a microscope. Fresh peduncles were submerged in clearing solution (80 g chloral hydrate in 30 ml water) until they became translucent.

Artificial model experiment. Movement was observed in a rotating artificial female floral bud and peduncle. The parts used to construct the artificial model are shown in Fig. S3e. The artificial model was designed to be three times the size of a natural *Vallisneria* female flower bud and peduncle. The bud shape mimicked that of *Vallisneria* as shown in the inset of Fig. 1c–3. Rotation was generated using a low-speed magnetic stirrer (7 s per rotation), for which stirring was fixed in a clockwise direction. Peduncle elongation was simulated by draining water at the rate of 2.5 cm min⁻¹ during experiments. Movies were recorded in two directions: side-view and upper-view. To adjust the direction of circumnutation, movies of the artificial model were converted into mirror images using AVS Video Editor Ver. 6.1.2.222 (Online Media Technologies Ltd, London, UK). The trajectory of the artificial flower tip was traced directly from the upper-view movie with ×0.5 playback speed. The amplitude of circumnutation was measured from the trajectory. The number of rotations and time required to complete a single rotation (single rotation time) were measured at ×0.5 playback speed.

1. Darwin, E. in *The Botanic Garden, a Poem. Part II. The Loves of the Plants.* (J. Johnson, London, 1791).
2. Darwin, C. & Darwin, F. in *The Power of Movement in Plants* (John Murray, London, 1880).



3. Israelsson, D. & Johnsson, A. A theory for circumnutations in *Helianthus annuus*. *Physiol. Plant.* **20**, 957–976 (1967).
4. Brown, A., Chapman, D. K., Lewis, R. F. & Venditti, A. L. Circumnutation of sunflower hypocotyls in satellite orbit. *Plant Physiol.* **94**, 233–238 (1990)
5. Johnsson, A., Solheim, B. G. B. & Iversen, T.-H. Gravity amplifies and microgravity decreases circumnutations in *Arabidopsis thaliana* stems: results from a space experiment. *New Phytol.* **182**, 621–629 (2009).
6. Hatakeda, Y. *et al.* Gravitropic response plays an important role in the nutational movements of the shoots of *Pharbitis nil* and *Arabidopsis thaliana*. *Physiol. Plant.* **118**, 464–473 (2003).
7. Kitazawa, D. *et al.* Shoot circumnutation and winding movements require gravisensing cells. *Proc Natl Acad Sci U S A.* **102**, 18742–18747 (2005).
8. Kiss, J. Z. Plants circling in outer space. *New Phytol.* **182**, 555–557 (2009).
9. Stolarz, M. Circumnutation as a visible plant action and reaction. Physiological, cellular and molecular basis for circumnutations. *Plant Signal Behav.* **5**, 380–387 (2009)
10. Caré, A.-F., Nefed'ev, L., Bonnet, B., Millet, B. & Badot, P.-M. Cell elongation and revolving movement in *Phaseolus vulgaris* L. twining shoots. *Plant Cell Physiol.* **39**, 914–921 (1998).
11. Nakajima, K., Kawamura, T. & Hashimoto, T. Role of the *SPIRAL1* gene family in anisotropic growth of *Arabidopsis thaliana*. *Plant Cell Physiol.* **47**, 513–522 (2006).
12. Buda, A., Zawadzki, T., Krupa, M., Stolarz, M. & Okulski, W. Daily and infradian rhythms of circumnutation intensity in *Helianthus annuus*. *Physiol Plant* **119**, 582–589 (2003).
13. Niinuma, K., Someya, N., Kimura, M., Yamaguchi, I. & Hamamoto, H. Circadian rhythm of circumnutation in inflorescence stems of *Arabidopsis*. *Plant Cell Physiol.* **46**, 1423–1427 (2005).
14. Kutschera, U., Siebert, C., Masuda, Y. & Sievers, A. Effects of submergence on development and gravitropism in the coleoptile of *Oryza sativa* L. *Planta* **183**, 112–119 (1990).
15. Wylie, R. B. The pollination of *Vallisneria spiralis*. *Bot. Gaz.* **63**, 135–145 (1917).
16. Kausik, S. B. Pollination and its influence on the behavior of the pistillate flower in *Vallisneria spiralis*. *Am. J. Bot.* **26**, 207–211 (1939).
17. Cox, P. A. Hydrophilous pollination. *Annu. Rev. Ecol. Syst.* **19**, 261–280 (1988).
18. Cox, P. A. Water-pollinated plants. *Scientific American* **269**, 68–74 (1993).
19. Darwin, C. in *The Movements and Habits of Climbing Plants*. (John Murray, London, 1875).
20. Sachs, J. & Ward, H. M. in *Lectures on the Physiology of Plants* (Clarendon, Oxford, 1887).
21. Gerbode, S. J., Puzey, J. R., McCormick, A. G. & Mahadevan, L. How the cucumber tendril coils and overwinds. *Science* **337**, 1087–1091 (2012).
22. Goriely, A. & Tabor, M. Spontaneous helix hand reversal and tendril perversion in climbing plants. *Phys. Rev. Lett.* **80**, 1564–1567 (1998).
23. Swarup, R. *et al.* Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nat. Cell Biol.* **7**, 1057–1065 (2005).
24. Tasaka, M., Kato, T. & Fukaki, H. The endodermis and shoot gravitropism. *Trends Plant Sci.* **4**, 103–107 (1999).
25. Morita, M. T. Directional gravity sensing in gravitropism. *Annu. Rev. Plant Biol.* **61**, 705–720 (2010).
26. Furutani, I. *et al.* The *SPIRAL* genes are required for directional control of cell elongation in *Arabidopsis thaliana*. *Development* **127**, 4443–4453 (2000).
27. Hashimoto, T. Molecular genetic analysis of left-right handedness in plants. *Philos. Trans. R. Soc. London Ser. B* **357**, 799–808 (2002).
28. Weizbauer, R., Peters, W. S. & Schulz, B. Geometric constraints and the anatomical interpretation of twisted plant organ phenotypes. *Frontiers in Plant Sci.* **2**, 62 (2011).
29. Kaul, R. B. Evolution and adaptation of inflorescences in the Hydrocharitaceae. *Am. J. Bot.* **57**, 708–715 (1970).
30. Svedelius, N. On the life history of *Enalus acoroides*. *Annals Roy. Bot. Gard. Peradeniya* **2**, 267–297 (1904).
31. Cunnington, H. M. XVI. Anatomy of *Enalus acoroides* (Linit.f), *Zoll. Trans. Linn. Soc. London, Bot.* **7**, 355–372 (1912).
32. Brown, A. H. Circumnutation: from Darwin to space flights. *Plant Physiol.* **101**, 345–348 (1993)
33. Bowling, A. J. & Vaughn, K. C. Gelatinous fibers are widespread in coiling tendrils and twining vines. *Am. J. Bot.* **96**, 719–727 (2009).
34. Meloche, C. G., Knox, J. P. & Vaughn, K. C. A cortical band of gelatinous fibers causes the coiling of redvine tendrils: A model based upon cytochemical and immunocytochemical studies. *Planta* **225**, 485–498 (2007).
35. Qiming, X., Haidong, C., Huixian, Z. & Daqiang, Y. Chemical composition of essential oils of two submerged macrophytes, *Ceratophyllum demersum* L. and *Vallisneria spiralis* L. *Flavour Fragr. J.* **21**, 524–526 (2006).
36. Gao, Y.-N. *et al.* Phenolic compounds exuded from two submerged fresh water macrophytes and their allelopathic effects on *Microcystis aeruginosa*. *Pol. J. Environ. Stud.* **20**, 1153–1159 (2011).
37. Inoue, N. *et al.* Ecological significance of root tip rotation for seedling establishment of *Oryza sativa* L. *Ecol. Res.* **14**, 31–38 (1999).
38. Lovett-Doust, J. & Laporte, G., Population sex ratios, population mixtures and fecundity in a clonal dioecious macrophyte *Vallisneria americana*. *J. Ecol.* **79**, 477–489 (1991).
39. Sullivan, G. & Titus, J. E. Physical site characteristics limit pollination and fruit set in the dioecious hydrophilous species, *Vallisneria americana*. *Oecologia* **108**, -285–292 (1996).

Acknowledgments

We thank Drs. Hideki Takahashi, Tokushiro Takaso, Toshio Hara, Katsushi Fukuyama, Yasunori Maekawa, Motoharu Okamoto, and Yasuro Kadono for their valuable suggestions; Fumio Sakuma for technical assistance with projection; and Yasutaka Kosuge for assistance with the model experiment.

Author contributions

K. Kosuge and S.I. recorded plant movement and wrote the paper; K. Kosuge constructed the nonliving model; K. Katou and T.M. analysed the movie images. All authors discussed the results and edited the manuscript.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

License: This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/>

How to cite this article: Kosuge, K., Iida, S., Katou, K. & Mimura, T. Circumnutation on the water surface: female flowers of *Vallisneria*. *Sci. Rep.* **3**, 1133; DOI:10.1038/srep01133 (2013).